UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Effect of blood on physical-chemical properties and discoloration of experimental silicate cement and MTA HP

Efeito do sangue nas propriedades físico-químicas e escurecimento de cimento de silicato experimental e do MTA HP

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Orientador: Prof. Dr. Marco AntonioHungaro Duarte

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“Se a tranquilidade da água permite refletir as coisas, o que não poderá a tranquilidade do espírito?”

Chuang Tzu

RESUMO

Efeito do sangue nas propriedades físico-químicas e escurecimento de cimento de silicato experimental e do MTA HP

Objetivo foi analisar radiopacidade, tempo de presa, escoamento, espessura, pH, solubilidade, liberação de íons cálcio, liberação de oxido nítrico – NO, descoloração dental e resistência à compressão de um cimento experimental (CE) de silicato de cálcio, com radiopacificadores (tungstato de cálcio, óxido de zircônio e sulfato de bário) e o MTAHP, e o efeito do contato com o sangue. Em 6 grupos: I – Cimento Portland; II – CE com 30% de tungstato de cálcio e 10% de sulfato de bário; III – CE com 20% de tungstato de cálcio e 20% de sulfato de bário; IV – CE com 30% de tungstato de cálcio e 10% óxido de zircônio; V – CE com 20% de tungstato de cálcio e 20% de óxido de zircônio e VI – MTAHP. O líquido do CE eral 30% de glicerina e 70% água destilada. Na radiopacidade, escoamento e espessura de filme a norma ISO 6876/2001 foi empregada. No tempo de presa a norma ASTM C266/08 e ISO 6876/2001. O escoamento e espessura norma ISO 6876/2001 (ISO, 2001). Para pH, solubilidade e liberação de íons cálcio, dentes de acrílico com cavidades retrógradas, n.10 com 5 ao sangue e imersos em água destilada. O pH e a liberação de íons cálcio nos períodos de 3h, 7h, 24h, 72h, 168h e 360h. Alteração volumétrica em Micro CT tempo inicial e 7 dias. A produção de NO pela reação de Griess. A espectrofotometria pela INTERNATIONAL COMMISION ON ILLUMINATION (1978), nos períodos de 7, 15, 30 e 60 dias. Na resistência à compressão emregou a norma ISO 1997 (2007). Os resultados mostraram que os cimentos experimentais apresentaram radiopacidade acima de 3mm Al, tempo de presa prolongado e sem interferência com sangue, escoamento maior e menor espessura de filme em comparação ao MTAHP, alteração volumétrica similar em relação ao MTAHP. O pH e a liberação íons cálcio discretamente maiores para o cimento MTAHP. A citotoxicidade aceitável, vez que os níveis dessas alterações são discretos. Espectrofotometria o G2 e MTAHP apresentaram leve alteração significativa e boa resistência à compressão e sem interferência do sangue. Os cimentos experimentais apresentaram propriedades adequadas para serem usados.

Palavras-chave: Cimento Portland. MTA. Biocompatibilidade.

ABSTRACT

Effect of blood on physical-chemical properties and discoloration of experimental silicate cement and MTA HP

The aim of this study was to analyze a Portland cement-based experimental cement with radiopacifiers (calcium tungstate, zirconium oxide and barium sulfate) and the HPMTA radiopacity, setting time, thickness, flow, pH, volumetric change, calcium ion release, release of nitric oxide – NO, tooth discoloration and compression strength, the effect when in contact with blood. In 6 groups: I (CS) - Portland Cement; II (CSCT1) - 60% Portland cement + 30% Calcium tungstate and 10% barium sulphate; III (CSCT2) - 60% Portland cement + 20% Calcium tungstate and 20% barium sulphate; IV (CSCT3) - 60% Portland cement + 30% Calcium tungstate and 10% Zirconium oxide; V (CSCT4) - 60% Portland cement + 20% Calcium tungstate and 20% Zirconium oxide; VI – HPMTA. 30% glycerin added to the distilled water. The radiopacity, ISO 6876/2001 (ISO, 2001), n.10 with 5 blood. The settling time standard ASTM C266/08, n.6 with 3 to blood. The flow and thickness standard ISO 6876/2001 (ISO, 2001). For pH, solubility and release of calcium ions, acrylic teeth with retrograde cavities, n.10 with 5 to blood and immersed in distilled water. The pH and release of calcium ions in the periods of 3h, 7h, 24h, 72h, 168h and 360h. Volumetric change in Micro CT initial time and 7 days. The production of NO by the Griess reaction. The spectrophotometry by INTERNATIONAL COMMISION ON ILLUMINATION (1978), 10 bovine teeth, periods of 7, 15, 30 and 60 days. Compression strength ISO 1997 (2007), n.12 with 6 to blood. It resulted in radiopacity within the recommended or above, a longer setting time and without interference with blood, higher flow and lower thickness compared to HPMTA, similar volumetric change compared to HPMTA. The pH and calcium release were slightly higher for the HPMTA cement. Acceptable cytotoxicity since the levels of these changes are so discrete. Spectrophotometric the CSCT1 and HPMTA presented slight significant change and the compression strength good resistance without interference to blood. The experimental cements had adequate properties to be used.

Key-words: Portland cement. MTA. Biocompatibility.

LISTA DE ABREVIATURA E SIGLAS

%	Porcentagem
x	Vezes
<	Menor
>	Maior
=	Igual
º	Grau
+	Mais
ADA	American Dental Association
Al	Alumínio
ASTM	American Society for Testing and Materials
CE	Cimento Experimental
CFU	Unidades Formadoras de Colônias
cm	Centímetro
G	Grupo
g	Gramas
h	Horas
ISO	International Organization for Standardization
KV	Quilovolts
L	Litros
microCT	Microtomografia Computadorizada
min	Minutos
mg	Miligrama
mL	Mililitro
mm	Milímetro
mm^3	Milímetro Cúbico
MDM	Derivados de Monócitos Humanos
MTA	Agregado Trióxido Mineral
MTA-HP	Agregado Trióxido Mineral HP
n	Número
NO	Óxido Nítrico

P	Significância Estatística
PBS	Solução salina tamponada com fosfato estéril
ppm	Partes por Milhão
µL	Microlitro
µm	Micrômetro
μm^2	Micrômetro Quadrado

SUMMARY

1 INTRODUCTION

1 INTRODUCTION

The mineral aggregate trioxide (MTA) is formed by calcium, silicon, aluminum and bismuth ions (CAMILLERI et al., 2005). The ions are in the form of tricalcium silicate, dicalcium silicate, tricalcium aluminate and tricalcium aluminoferrite (CAMILLERI, 2008a). In this way, MTA has become a calcium silicate cement and there is a chemical similarity between the MTA and a cement based on calcium silicate, Portland cement, used in civil construction (ESTRELA et al., 2000). Studies have shown similarity in the constitution of both, which differ only by the presence of bismuth oxide in the MTA (CAMILLERI et al., 2005). As for the physical properties, they presented similar results (ISLAM, CHNG, YAP, 2006) and also biological (SAIDON et al. 2003; DE DEUS et al., 2005). Some characteristics, despite having good physical, chemical and biological properties, may limit the use of MTA, such as high cost, low flow and possibility of staining of dental structures (BELOBROV; PARASHOS, 2011; DUARTE et al., 2012).

The widely used MTA in Endodontics as a retroburst material, pulp cap and perforator sealer (LEE et al., 1993; TORABINEJAD et al., 1995; TANOMARU-FILHO et al., 2006). This material exhibits adequate biological properties, such as the ability to promote periapical tissue repair (TORABINEJAD et al., 1995; HOLLAND et al., 2001c), antibacterial and antifungal properties (PARIROKH; TORABINEJAD, 2010) and inhibition of the growth of microorganisms (JACOBOVITZ et al., 2009). Due to satisfactory biological properties, the MTA has been proposed to be used as a base for root canals (JACOBOVITZ et al., 2009; SCARPARO et al., 2010, OLIVEIRA et al., 2011; CAMILLERI et al., 2011). Some characteristics of the MTA, however, make it difficult to use as root canal sealer cement, such as its high cost and handling characteristics (JACOBOVITZ et al., 2009). Portland cement presents composition and physico-chemical and biological properties similar to the MTA, with a reduced cost (WUCHERPFENNIG et al., 1999; ESTRELA et al, 2000; HOLLAND et al., 2001c; SAIDON et al., 2003). Thus, Portland-based experimental cements have been developed in an attempt to make the material more accessible without, however, losing its satisfactory physico-chemical and biological properties.

The sandy consistency and the low viscosity of the MTA make it difficult to use as a sealant. Both MTA and Portland do not have enough flow to be used as root canal sealer cements (JACOBOVITZ et al., 2009), thus, it is necessary to add components that confer greater flow to these cements. Propylene glycol and distilled water have been used as vehicles for the purpose of imparting higher viscosity to MTA or Portland-based cements (HOLLAND et al., 2007; SCARPARO et al., 2010). In 2009, Camilleri (2009) verified that the addition of water and a water-soluble polymer to the MTA resulted in a viable material to be used as a root canal sealant cement and did not cause changes in the hydration characteristics of the material. In 2011, this experimental cement was evaluated by Camilleri and Mallia in relation to setting time, dimensional change, fluid absorption, microstructure and porosity. The authors verified that the new cement presented a suitable setting time, stable dimensions, besides fluid absorption and porosity inferior to the MTA. In the same year, (CAMILLERI et al., 2011a) evaluated the sealing of this same cement and verified that it was similar to the cement Pulp Canal Sealer. The authors also observed that the experimental cement was able to release calcium ions. Also in 2011, (OLIVEIRA et al., 2011) evaluated an experimental cement (MTAS) composed of Portland, a radiopacifying agent, additives and a vehicle (not specified by the authors) in relation to sealing and bacterial infiltration. MTAS, however, showed a higher bacterial infiltration compared to AH Plus and Sealapex.

The absence of radiopacity from Portland cement requires the addition of a radio-opacifying agent to allow its clinical use (DUARTE et al., 2009), both as a retrobuturing cement and as a root canal obturator. The MTA based cements present in their composition the radiopacifier bismuth oxideIt has been reported that bismuth oxide may interfere with the physicochemical properties of MTA and Portland cement, affecting the hydration process (CAMILLERI, 2008a, b), increasing porosity and decreasing resistance to compression force (COOMARASWAMY, LUMLEY, HOFMANN, 2007). In addition, one study demonstrated that MTA may cause dental dimming (BELOBROV et al., 2011), which is undesirable for a root canal sealer cement. It is suspected that this fact may be related to the presence of bismuth oxide in its composition. Alternative radiopacifiers have been studied to replace bismuth oxide in the composition of the MTA cement and to be added to Portland (DUARTE et

al., 2009; CAMILLERI, 2010a, b, CAMILLERI, CUTAJAR, MALLIA, 2011b; CUTAJAR et al., 2011).

Calcium tungstate is one of the radiopacifiers proposed to replace bismuth oxide. Calcium tungstate, also known as chileite (MERCK INDEX, 1996), is a fine white powder produced by the stoichiometric mixture of calcium oxide, calcium carbonate and tungsten acid. This substance is used in the treatment of tumors and endodontics as one of the components of AH Plus (LEYHAUSEN et al., 1999). Gomes-Cornelio et al. (2011) did not verify cytotoxicity for calcium tungstate and zirconium oxide, suggesting that these agents may be good alternatives for use with Portland cement. However, the effect of the addition of these agents on a root canal sealer is still little explored. Thus, the evaluation of a Portland-based cement associated with different concentrations of this alternative radiopacifying agent in relation to the physical, chemical and biological properties becomes timely.

MTA has low or almost no solubility, which is attributable to the addition of bismuth oxide (RAO, RAO, SHENOY, 2009; PARIROKH, TORABINEJAD, 2010), as well as alkaline pH, which is a significant chemical property because it can help repair, stimulating the mineralization process (HOLLAND et al., 2002). The solubility of a sealant cement is undesirable, since the dissolution of the material may allow bacterial infiltration, compromising the treatment. Typically, ISO 6876 specifications (FRIDLAND, ROSADO, 2005; VIVAN et al., 2010) or ANSI/ADA 57/2000 (HUNGARO DUARTE, MINOTTI, RODRIGUES, 2012) are adopted to determine solubility. However, as proposed in the Cavenago et al. (2014), study a methodology was performed to evaluate the volumetric solubility of the MTA, using computerized microtomography to produce the volumetric data, and this new methodology better simulates the clinical situation.

Cytotoxicity tests in cell culture are considered initial, since they determine in a preliminary way the behavior of the materials under test. In this type of study, cells isolated in culture medium are evaluated in relation to cell death when exposed to a certain material. Several studies have evaluated the cytotoxicity of MTA and Portland cements (SAIDON et al., 2003; DE DEUS et al., 2005; HWANG et al., 2009). Thus, with the importance of the biological properties of endodontic cements, such as the release of nitric oxide, since for tissue repair, both pulp and periapical, it is imperative

that the obturator materials do not stimulate an exacerbated inflammatory response, that is, promote change in the inflammatory process (AL-HIYASAT; TAYYAR; DARMANI, 2010).

Some studies, such as those by Nekoofar, Stone, Dummer (2010), have reported the influence of the effects of pure fresh human blood contamination on the strength to compression and surface microstructure of the MTA (Pro-Root®). The effect of human blood and serum on microhardness and surface microstructure of white and gray MTA in the short and long term was also evaluated.

The search for new materials with capabilities to meet the requirements of those already available in the market, is therefore essential for scientific advancement and consequently improvements in endodontic treatment. The absence of studies on the appearance of interference in physicochemical and biological properties (radiopacity, setting time, film thickness and flow, pH, volumetric change, calcium ion release, cytotoxicity with the release of nitric oxide when contact with macrophages, tooth discoloration and compressive strength), evaluating the effect when they come into contact with the blood of a Portland cement-based experimental sealer with different radiopacifiers (Calcium Tungstate, Zirconium Oxide and Barium Sulphate) and the HPMTA.

Therefore, the study was carried out in three stages:

1. Effect of the immersion moment at the volumetric alteration of the Mineral Trioxide Aggregate HP.

2. Analysis of the solubility, pH, calcium release ions in contact with blood and citotoxicity of experimental and comercial calcium silicate cements.

3. Comparison of the physical properties of experimental Calcium Silicate based cement with different radiopacifiers and contac MTA HP when in contact with the blood.

2 ARTICLES

2 ARTICLES

The article presented in this Thesis was written and submited according to the Dental Press Endodontics

2.1 ARTICLE 1 – Effect of the immersion moment at the volumetric alteration of the Mineral Trioxide Aggregate HP

TÍTTULO: Efeito do momento de imersão na alteração volumétrica do Agregado Trióxido Mineral HP.

ABSTRACT

Solubility is a property related to the dissociation of the constituents of the material by the action of contact with the surrounding liquid, for this reason, the aim of this study was to evaluate the effect of the hydration during the scanning in the microtomography on the volumetric alteration of the MTA HP. Twenty acrylic teeth upper incisor with retrograde cavity were utilized. The MTA HP cement was inserted into the cavity using a Paiva condensor. The specimens were visually inspected with a 5x magnifying glass to ensure they did not remain void or gaps. The specimens were divided into 2 groups (n=10). The teeth were scanned shortly after handling the MTA. For the group with water immersion, Eppendorf was kept with 1mL the water during the scanning and the other group, the teeth were scanning without water. In the two groups the teeth were immersed into water during 7 days after scanning. Next the teeth were newly scanned in the Micro-CT using the same parameters and condtions of each group. Reconstruction of images by the Nrecon software and the solubility volume determined by the CTan, analyzing the volumetric change. The group of specimens scanned immersed into the water presented higher volumetric change with statistically significant differences in relation the group scanned without immersion. The scanning of the specimen immersed in water favors the greater volumetric loss of the material. Studies to evaluate volumetric change of calcium silicate cements should be made immersed in water.

Key-Words: Solubility, Root Canal Obturation, Physical and Chemical Properties.

RESUMO

A solubilidade é uma propriedade relacionada à dissociação dos constituintes do material pela ação de contato com o líquido circundante, por isso, o objetivo deste estudo foi avaliar o efeito da hidratação durante o escaneamento na microtomografia sobre a alteração volumétrica do MTA HP. Incisivos superiors em acrílico com cavidade retrógrada foram utilizados. O cimento MTA HP foi inserido na cavidade utilizando um condensador de Paiva. Os espécimes foram visualmente inspecionados com uma lente de aumento de 5x para garantir que não permanecessem vazios ou lacunas. Os espécimes foram divididos em 2 grupos (n = 10). Os dentes foram digitalizados logo após o manuseio do MTA. Para o grupo com imersão em água, Eppendorf foi mantido com 1mL de água durante o escaneamento e o outro grupo, os dentes foram escaneados sem água. Nos dois grupos os dentes foram imersos em água durante 7 dias após o escaneamento. Em seguida, os dentes foram novamente digitalizados no Micro-CT usando os mesmos parâmetros e condições de cada grupo. Reconstrução de imagens pelo software Nrecon e o volume de solubilidade determinado pelo CTan, analisando a variação volumétrica. O grupo de espécimes escaneados imersos na água apresentou maior alteração volumétrica com diferenças estatisticamente significantes em relação ao grupo escaneado sem imersão. O escaneamento do espécime imerso em água favorece a maior perda volumétrica do material. Estudos para avaliar a alteração volumétrica de cimentos de silicato de cálcio devem ser imersos em água.

Palavras-Chave: Solubilidade, Obturação do Canal Radicular, Propriedade Físicas e químicas.

INTRODUCTION

Several studies have analyzed the physicochemical properties of endodontic and retrograde filling materials (1,2,3,4,5). Recently a study correlated the results of physicochemical properties usually obtained by standard tests with the results obtained by tests using new methodologies such as computerized microtomography (6).

The proposed tests with Micro-CT complemented the conventional tests, allowing three-dimensional data to be obtained, perfecting the tests recommended by ISO standards and representing standardized and reproducible methods (1-4,7). Generally, in vitro tests evaluating the properties of solubility and volumetric change

have been done according to the specifications of ANSI / ADA n57 or ISO 6876.These standards are based on the difference between the weights before and after the placement of the cement in distilled water (5,8,9,10,11). However, this study followed the methodology based on the volumetric change obtained from the digitalization of three-dimensional images of the specimens obtained by micro-CT (5,12). This methodology has a great advantage because it evaluates only the radiopaque material, thus excluding the water under analysis, which is a factor that must be considered, since MTA is hydrophilic cement. The specimens simulated the clinical aspect in better shape and allowed the data to be compared with those of other studies that have used retrograde material in cavities of replicas of natural teeth (4,12,13).

Solubility is a property directly related to the dissociation of the constituents of the material by the action of contact with the surrounding liquid. Thus the larger contact area, as found in samples tested according to the ISO standard, increases the possibility of greater solubility. Furthermore, analysis performed after periods of immersion in distilled water complements evaluation of the solubility of the materials, and allows greater understanding of the dimensional behavior and solubility of the materials in periods longer than 24 hours (7,14). As shown in the studies that performed the solubility or volumetric change tests using blocks with retrograde cavities, the teeth were scanned by computerized microtomography (micro-CT) but without being in contact with water, and normally the material heave the setting without coming into contact with humidity.

The MTA cement is formed by calcium, silicon, aluminum and bismuth ions. The ions are in the form of tricalcium silicate, dicalcium silicate, tricalcium aluminate and tricalcium aluminoferrite. As a result, the MTA has become a calcium silicate based cement. Widely used this material in parendodontic surgeries, apitifications, pulp capping, sealing of perforations and pulpotomias (15).

However, these studies did not evaluate the solubility or volumetric change during the setting time of the material. Note that no studies were found, which evaluated whether scanning with the teeth immersed in water interfered in the volumetric change of the material. Therefore, the aim of this study was to verify and compare whether scanning the specimens immersed in distilled water would influence in the volumetric change (solubility), thereby reproducing a situation closer to that of the surgical or perforation site, due to local humidity at all times.

METHODOLOGY

Obtaining Specimens

The white MTA HP cement (Angelus, Londrina, PR, Brazil) was mixed using the proportion of 3 parts of the powder and 1 part of distilled water. Twenty acrylic teeth, replicas of a central maxillary incisor were made and retrograde cavities with 1012 standardized spherical drill bits with 3 mm of deep were performed and filled with MTA cement using an operating device such as the Paiva condenser. The specimens were visually inspected with a 5x magnifying glass to ensure they did not contain void or gaps. The specimens were divided into 2 groups (n = 10) according to the immersion in water, or not during the scanning procedure by Micro CT.

Solubility analysis

The teeth were scanned shortly after handling the MTA. Group 1 was kept in hydrated Eppendorfs with 10ml with 10 mL distilled water during the scanning, while the replicas of Group 2 were scanned without immersion in the water. The solubility was measured volumetrically by a microcomputer from the tomographic images (10). The samples were scanned using a microfocus CT scanner (SkyScan 1174v2; SkyScan, Kontich, Belgium). The scanning parameters were determined using 50 kV of X-ray tube voltages, 800 μA of anode current, a voxel size of 14.1 μm with 1,1 ° pitch of rotation and 360 ° rotation. Digital data with 1024 x 1304 pixels were compiled by the reconstruction software (NReconv1.6.4.8, SkyScan), and the software (CTanCTan v1.11.10.0, SkyScan) was used for volume measurements. For each sample, a binary value was adjusted and through the 3D plug-in the analysis the total volume (mm3) was recorded. After the first readout, the samples were immersed in glass vials containing 10 mL of distilled water and then stored at 37 °C for 168 h. Subsequently, the samples were scanned again using exactly the same parameters and conditions as those used in the first scan, and the volume was analyzed again. The results were converted into solubility percentages. The values were subjected to the Shapiro-Wilks test to evaluate the normal distribution. Due to absence of normal distribution, the intra-group comparison was made by using the Wilcoxon test, and for comparison of the percentage of volumetric change between the groups, the Man-Whitney test was used. The significance level was 5%.

RESULTS

The two groups presented statistically significant differences between the initial and final volume ($P<0.05$) in Table 1.

The group scanned immersed in the water presented higher volumetric change values with statistically significant differences in comparison with the group scanned without immersion in water. Figure 1 shows the representation of the three-dimensionalsolubility reconstructions after the initial and final volume micro-CT scans of Group 1 immersed in water and of Group 2 without immersion in water.

DISCUSSION

The volumetric change of root end and perforation sealing materials is an important property, since dissolution of the material can allow infiltration, compromising the success of the treatment.

In vitro tests evaluating the properties of solubility or volumetric change have used the ANSI / ADA n57 or ISO 6876 specifications, which are based on the difference between the weights before and after the placement of the cement in distilled water (1-3,7,10). However, hydrophilic material can absorb water and increase in weight without adequately demonstrating its solubility. Another point is that in the cited methodology, the material is tested after setting and it does not demonstrate the solubility during setting.

The present study followed the methodology based on the volumetric change obtained from the digitalization of three-dimensional images of the specimens by micro-CT (5,10,11). This methodology has a great advantage because it only evaluates the radiopaque material, thus excluding the water under analysis, which is a factor that must be considered because MTA is a hydrophilic cement. Another point is that the specimens used presented similarity with the clinical aspect, allowing the data to be to compare with those of other studies that used the root end filling material in cavities in replicates of natural acrylic teeth (3,12,13).

New methodologies such as computerized microtomography can be used to analyze physical-chemical properties of endodontic types of cement and root end filling materials as well dimensional change (11-13, 16).

The use of microcomputed Tomography (Micro-CT) in the present study allowed the volumetric analysis (in mm^3) of the materials, and showed the dimensional change. Furthermore, analysis after the periods of immersion in distilled water complemented

evaluation of the solubility of the materials and allowed greater understanding of the dimensional behavior and solubility of the materials in periods longer than 24 hours. The images were acquired using microtomography, allowing the use of the same specimen in different periods of analysis. The protocols developed using MicroCT to evaluate the filling capacity of the materials allowed a three-dimensional analysis of the filling percentage from the empty cavity (16).

However, in the other studies (12,17-23) the scanning was performed with the material without it coming into contact with the liquid. Thus during the first hour, which is the time taken to perform scanning, the material was without contact with moisture and consequently it did not have the effect of solubilization. Hydration of the MTA cements and calcium silicate results in reduction of the space for solubilization and precipitation of hydrated compounds. There is growth and consequent densification of the matrix of these products, probably due to the precipitated surface crystals that are released from di- and tricalcium silicate-containing materials, and these avoid a further increase in solubility (11,15,24,25). Porosity occurs because of spaces in the non-hydrated cement (24), and the porosity and solubility of materials may affect their stability, integrity, and durability (25). The porosity may be visually determined by observing the size and distribution of pores in the polished surface of cements (11). However, comparing the volumetric change with hydration and processing the actual state is important.

The aim of this study was to verify and compare the volumetric change during the scanning procedure by micro-CT with the specimens immersed or not in distilled water. The results of the present study showed that the scanning of the material immersed in water significantly increased the volumetric loss of the material, demonstrating that the higher volumetric change values occurred during the setting of the MTA HP. Probably the scanning with the specimen immersed in water favored greater reactivity and formation of Portland (Calcium hydroxide), which is a soluble component (15).The majority of the studies in the literature were conducted without the ideal reproduction of moisture, which influenced the solubility test, therefore it is suggested that in new studies that will be using this methodology, the specimens should be immersed in water during the scanning procedure.

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FIGURE LEGENDS

Figure 1

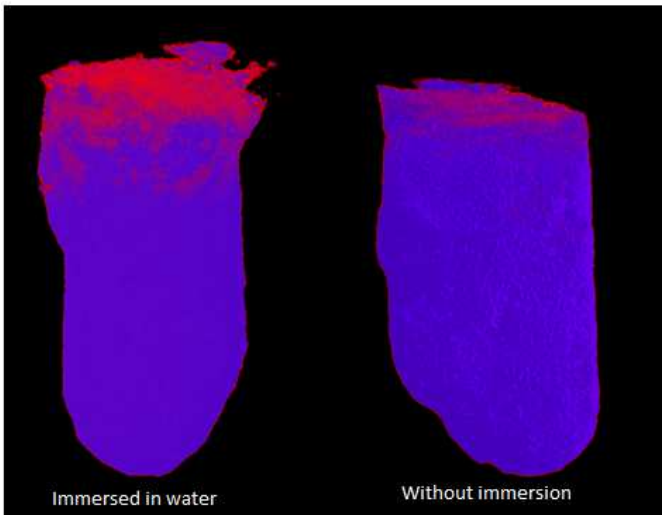
Shows the representation of the three-dimensional solubility reconstructions after the initial and final volume micro-CT scans of Group 1 immersed in water and of Group 2 without immersion in water.

Table 1 – Median, minimum and maximun values of the initial volume, final volume and percentage solubility after 7 days in the studied groups.

	Initial volume	Final Volume	% Solubility
Waterimmersion	0.52 (0.21-0.94)[a]	0.39 (0.06-0.70)[b]	23.5 (0-70.07)[A]
Withoutwaterimmersion	1.43 (0.16-1.83)[a]	1.33 (0.16-1.77)[b]	1.55 (0-13.73)[B]

Different lowercase letters show the statistically significant difference between the initial and final volumes in the intra-group comparison (P<0.05). Different uppercase letters show statistical significant differecen at the percentage of volumetric alteration in the comparison between the groups.

Figure 1



2.2 ARTICLE - Effect of the contact with blood in the volumetric alteration, pH, calcium release ions of experimental and comercial calcium silicate semente and analysis of their citotoxicity.

The article presented in this Thesis will be written according to the International of Endodontics

Abstract
Contamination by blood and tissue fluids of repair cements is usual in a clinical environment in which moisture is always present. The objective of this study was to analyze the alteration of experimental Calcium silicate based cement and the MTA HP for volumetric alteration, pH and calcium ions release when they come into contact with water and blood, as well as to evaluate the cytotoxicity with release of nitric oxide (NO) when in contact with macrophages. The cements were divided into 6 groups: I – Calcium silicate (CS); II – Calcium Silicate with 30% calcium tungstate and 10% barium sulfate (CSCT1); III – Calcium Silicate with 20% calcium tungstate and 20% barium sulfate (CSCT2); IV – Calcium Silicate with 30% calcium tungstate and 10% zirconium oxide (CSCT3); V – Calcium Silicate with 20% calcium tungstate and 20% zirconium oxide (CSCT4) and VI - MTA HP (MHP). To determine the pH, volumetric alteration and release of calcium ions release, acrylic replicas teeth with retrograde cavities were used and filled with the cements and placed in contact with the blood and immersed in distilled water. The pH and calcium ions release were in the periods of 3h, 7h, 24h, 72h, 168h and 360h. The alteration volumetric was analyzed volumetrically in Micro CT at the initial time and after 7 days. NO production was obtained by measuring nitrite levels by the Griess reaction. The results obtained for solubility were considered acceptable without statistically significant difference between the materials ($P>0.05$). The largest increase in alkalinity occurred for the MHP. The higher calcium ions release by the MHP and CSCT4. The imersion in the blood did not affect the volumetric alteration and interfered at the pH and calcium. For the cytotoxicity, the materials presented low citotoxicity and were similar. It was concluded that the contact with the blood do not interfered in the properties of the materials. The materials presented similar alteration volumetric and citotoxicity in relation to the MTAHP. The MTAHP cement presented the highest pH and calcium ion release. For the experimental cements, the CSCT4 presented the higher calcium release.
Key-Words: MTA. Portland cement. Physicochemical properties.

INTRODUCTION

The repair cements during their use in endodontic surgery, pulpotomy, root perforation and in apicification occur the contact with the blood and tissue fluids. The blood, in contact with the material, becomes embedded and this contamination could present some effect on its physical, chemical and biological properties (Nekoofar *et al.* 2010, Shakouie *et al.* 2012). The MTA cement is formed by calcium, silicon, aluminum and bismuth ions (Camilleri *et al.* 2005). The ions are in the form of tricalcium silicate,

dicalcium silicate, tricalcium aluminate and tricalcium aluminoferrite(Camilleri 2008). As a result, the MTA has become a calcium silicate based cement. There is a chemical similarity between the MTA and other calcium silicate cements such as the Portland cement (Estrela *et al.* 2000). Studies have shown similarity in the constitution of both, differentiating only by the presence of bismuth oxide in the MTA (Estrela *et al.* 2000, Camilleri *et al.* 2005). The physical and bilological comportments of these materials were also similar properties, the results were similar (Saidon *et al.* 2003, De Deus *et al.* 2005, Islam *et al.* 2006). Some characteristics may limit the use of MTA such as high cost, low flowability and possibility of staining of dental structures (Belobrov & Parashos 2011, Duarte *et al.* 2012).

Thus, experimental of calcium silicate cements have been developed in an attempt to make the material more accessible without losing its satisfactory physical-chemical and biological properties and other substances have been associate to promote radiopacity and platicity of the materials (Hungaro Duarte *et al.* 2009, Marciano *et al.* 2013).

The pH and release of calcium ions important chemical properties because they help repair by stimulating the process of tissue mineralization (Holland *et al.* 2002). The solubility of a repair material is undesirable because the dissolution of the material may allow bacterial infiltration, compromising the treatment. Normally, ISO 6876 specifications (Fridland & Rosado 2005, Vivan *et al.* 2010) or ANSI / ADA 57/2000 (Hungaro Duarte *et al.* 2012) are adopted to determine solubility. However, calcium silicate cement are hydrophilic, the sorption of water can increase the weight, and the material can be lostingcomponente, favor false results. A methodology was proposed to evaluate the volumetric alteration of the MTA, using computerized microtomography to produce the volumetric data, and this new methodology simulates the clinical situation (Cavenago *et al.* 2014).

Cytotoxicity tests in cell culture are considered initial test because they preliminar determine the behavior of the materials under test. In this type of study, cells isolated in culture medium are evaluated in relation to cell death when exposed to a certain material. Several studies have evaluated the cytotoxicity of MTA and Portland cements (Saidon *et al.* 2003, De Deus *et al.* 2005, Hwang *et al.* 2009). Thus, with the importance of the biological properties of endodontic cements, such as the release of nitric oxide, since for tissue repair, both pulp and periapical, is imperative that the repair

materials do not stimulate an exacerbated inflammatory response (Al-Hiyasat *et al.* 2010).

The search for new materials with capabilities to meet the requirements of those already available in the market is essential for scientific advancement and consequently improvements in endodontic treatment. Thus, the proposal of the experimental cement based on Portland cement was to try to achieve better properties regarding solubility, pH, calcium ions release and release of nitric oxide compared to MTA-HP. Due to a few researches carried out in this scope it is suggested that more analyzes should be carried out to definitively prove the properties and effectiveness of these cements.

METHODOLOGY

The materials used in the analyzes were the MTA HP (Angelus, Londrina, Brazil), structural white Portland cement (Votorantim, São Paulo, Brazil), glycerine, calcium tungstate, zirconium oxide and barium sulfate. The materials will be divided into 6 (six) groups:

Group I (CS) - Portland cement + Liquid (70% distilled water and 30% glycerin)

Group II (CSCT1) - 60% Portland cement + 30% Calcium tungstate and 10% barium sulphate + Liquid (70% distilled water and 30% glycerin)

Group III (CSCT2) - 60% Portland cement + 20% Calcium tungstate and 20% barium sulphate + Liquid (70% distilled water and 30% glycerin)

Group IV (CSCT3) - 60% Portland cement + 30% Calcium tungstate and 10% Zirconium oxide + Liquid (70% distilled water and 30% glycerin)

Group V (CSCT4) - 60% Portland cement + 20% Calcium tungstate and 20% Zirconium oxide + Liquid (70% distilled water and 30% glycerin)

Group VI – (MHP) MTA HP.

For the distributions of the proportions of the experimental mixtures, a precision analytical balance was used, just as the blood used in the research was human blood collected in a specific laboratory with the consent of a voluntary donor.

Human Research Ethics Committee of State University of São Paulo (CAAE – 94400518.0.00005417) approved this study.

Volumetric alteration analysis

For the determination of the volumetric alteration of the cements, 60 acrylic replicas teeth with retrograde cavity were used. The retrograde cavities were filled with the cements according to the group (10 specimens by group) and so were divided into two subgroups, where 5 (five) specimens from each group came into contact with 1mL of blood and other 5 with 1mL of water. The specimens placed in eppendorfs with cotton and Hemospon® with water or blood, simulating a real clinical situation and then scanned by a micro CT scanner (1174v2 SkyScan, SkyScan, Kontich, Belgium), and immersed in eppendorfs again and taken to the oven at 37 °C where they remained during the experimental period. After 7 days, the specimens were newly scanned by means of Micro-C. The initial volume and after 7 days was measured. The volumetric alteration was calculated in percentage by the difference between the initial volume and the volume 7 days and divided by initial volume.

Determination of pH and release of calcium ions

For the determination of the pH of the cement variables, the same 60 acrylic teeth were used. The specimens were immersed in glass vials containing 10mL of ultra pure water. The evaluations were carried out in periods of 3, 7 and 15 days of immersion. After each period the specimens were with drawn from the flasks and immersed in new flasks containing the same volume (10mL) of ultra pure water for the then new period of time. PH was determined by means of a pH meter previously calibrated with solutions of known pHs (4, 7 and 14). For gauging, removed the specimen from the flask and was taken to a shaker for 5 seconds. After shaking, was puted in contact with the pH meter electrode.

The calcium ions release was carried out in the same periods used for the pH reading. Atomic absorption spectrophotometer equipped with a calcium-specific hollow cathode lamp was used. To prevent possible alkali metal interferences, lanthanum solution was prepared by diluting 9.8 g of lanthanum chloride in 250 ml of acid solution. A stock solution of calcium was prepared by diluting 2.4972 g of calcium carbonate in 50 ml of ultra pure water. To this solution was added dropwise 10 mL of concentrated hydrochloric acid. Subsequently diluted in 1000mL of distilled water. After preparation, 1mL of this preparation corresponded to 1mg of calcium. With this solution, was prepared the standard solutions of calcium, being: 20mg / L, 10mg / L, 5mg / L, 2,5mg / L, 1,25mg / L. In the reading, the 8mL of the samples' standards or water were

associated with 2mL of the lanthanum chloride solution. To bring the apparatus to zero absorbance, the nitric acid solution was used. Calculations of the release of calcium ions are made by means of the equation of the straight line of the standard curve.

Isolation and culture of human monocyte derived macrophages (MDM)

In this test just the experimental cements (CSCT1, CSCT2, CSCT3 and CSCT4) were tested. Heparinized whole blood (30 mL) was obtained from healthy volunteer 1 and peripheral blood monocytes were separated using Histopaque 1083 gradients (Sigma-Aldrich Brazil Ltda., São Paulo, Brazil). Immediately, 1 x 10 6 cells / mL were counted with a hemocytometer using neutral red and cultured in a 24-well culture dish containing sterile spherical glass coverslips (13 mm in diameter). After 2 hours in 5% CO2 at 37 ° C, the non-adherent cells were removed by aspiration. Finally, the monocytes were incubated in 1 ml of complete medium (RPMI 1640 + 10% heat inactivated fetal calf serum [FCS] + 1% penicillin / streptomycin). After 7 days, the viability of MDM was assessed by exclusion of trypan blue (> 94%).

Phagocytosis assay

To stimulate the groups with the macrophages the MDM was grown in a 24-well plate mixed with the experimental cements with a diluted ratio of 0.066 g for each 3 mL of liquid (0.15 microliters of glycerin and the remaining water), at 37 ° C for 30 minutes in 5% CO2 atmosphere. There after, the wells were washed three times with sterile phosphate buffered saline (PBS) to remove the extracellular components. Cells were fixed with 4% paraformaldehyde for 30 minutes; and finally washed twice with sterile PBS. Internalized cements were observed using a fluorescence extinction technique described in an earlier study (HED, 1977). Briefly, 0.05 mg / mL of acridine orange was used for 15 minutes to stain the groups of cements and MDM. Immediately, the fluorescence of the remaining extracellular clusters was extinguished by the crystal violet stain. Coverslips with adherent MDM were carefully removed and placed on glass slides using a mounting medium (VECTASHIELD®). The slides were analyzed by the Axiostar HBO + 50 / AC Fluorescence Mycroscopy (Carl Zeiss, Germany), and the groups of the phagocytized cements were quantified considering the number of each groups of internalized cements per cell (<5 and ≥5), with increase of 100x in 20 randomly selected fields. The results were obtained from the volunteer's blood samples.

Production of Nitric Oxide (NO)

MDM were mixed with the cements described above, and the supernatants recovered after 24 hours. NO production was obtained by measuring nitrite levels by the Griess reaction. The absorbance was measured in a spectrophotometer at 540 nm and the values expressed in μM.

Statistical Analysis

The results were submitted to Kolmogorov-Smirnov test for normal distribution. In function of absence of normality, the Kruskal-Wallis and Dunn tests were used. For all tests, the significance level of 5% was considered.

RESULTS

The values and statistical differences of the volumetric alteration of the cements evaluated in percentage are shown in Table 1. The largest alteration of volume found was for the cement of the CSCT4 in contact with the blood (28,19%) with statistically significant difference with the CSCT2 group in contact with blood (2,88%). For the 7 days period, the groups of the cements did not present statistically significant differences ($P>0.05$). In the intragroup comparison, only CSCT4 showed statistically significant difference ($P<0.05$) between the blood and distilled water.

The pH values of the water before of the immersion the material was 6.61. The results of the pH analysis tests shown in Table 2. In the 3 hour period the highest pH or alkalinity was found for MHP in contact with distilled water, being statistically significant ($P<0.05$) in relation to the cements of CSCT1 groups in contact with water, CSCT2 in contact with water and CSCT3 in contact with water and this with the lowest alkalinity for the period. For the period of 24 hours, the highest pH was for the MHP in contact with water, differing statistically significant with the cements of the same MHP but in contact with the blood, and with CSCT3 in contact with water. In the period of 72 hours, the MHP cement in contact with water was the one that obtained a higher pH, presenting a statistically significant difference with the cements of the same MHP but in contact with the blood. For the period of 168 hours, the highest pH was for the CSCT1 cement group in contact with the blood, with a statistically significant difference only with the CSCT1 cement with water, also representing the lower pH of the period. In the last period of time 360 hours, the highest pH occurred for the cement of the MHP

group in contact with water, presenting statistically significant differences with all other cement groups in contact with water or blood.

The results of the calcium ion release tests in mg / L are shown in Table 3. In the 3 hours period, the highest calcium ion release occurred in MHP in contact with distilled water. The lowest values occured with the CSCT3 in contact with water, CSCT2 in contact with water and with blood and CSCT1 in contact with water. For the 24 hours period, the highest release was for MHP in contact with blood, differing statistically significant to the CSCT2 and CSCT3 cement in contact with water. For the experimental cements, the highest calcium release occured to the CSCT4 in contact with water. In the period of 72 hours the CSCT4 cement in contact with water proprotionned the greatest calcium ion release, presenting statistically significant difference ($P<0.05$) with the cements of the groups CS, CSCT1 CSCT2 and CSCT3 in contact with water. For the period of 168 hours, the highest values of calcium ion release was for the CSCT4 group in contact with water with a statistically significant difference with the CS, CSCT1 CSCT2 and CSCT3 cements in contact with water. In the 360 hours period, the greatest calcium ion release occurred to the cement of the MHP group in contact with the blood, presenting statistically significant differences ($P<0.05$) with the cements of CSCT1 and CSCT2 in contact with water.

Table 4 contains the values of the percentage of nitric oxide release by macrofages for each experimental cement group. The results were that after 24 hours the groups showed decreased NO release between the groups. With exception CSCT3 which presentes values higher that postive control, the other cements presented similar values, although the differences were not statistically significant ($P>0.05$).

DISCUSSION

The proposal of this study was developa experimental cement based of Calcium Silicate and analyze the effect of the blood on the volumetric alteration, pH, calcium ion release of these materials, comparing with the MTAHP and compare the cytotoxicity of the experimental and MTAHP analyzing the nitric oxide.

To determine the pH, volumetric alteration and calcium ions release, acrylic replicas teeth with retrograde cavities were used. The retrograde cavities were filled according to the tested group and placed in contact with the blood and immersed in distilled water in one subgroup and without blood contact in the other subgroups. The pH and calcium ions released were determined in the periods of 3hs, 7hs, 24hs, 72hs,

168hs and 360hs. NO (nitric oxide) production was obtained by measuring nitrite levels by the Griess reaction. The volumetric alteration was analyzed in Micro CT at the initial time and after 7 days immersed in water. Normally, ISO 6876 specifications (Fridland & Rosado 2005, Vivan *et al.* 2010) or ANSI / ADA 57/2000 (Hungaro Duarte *et al.* 2012) are adopted to determine solubility. A new methodology was used to evaluate the volumetric alteration of the MTA, using computerized microtomography to better simulates the clinical situation (Cavenago *et al.* 2014). This methodology has the great advantage because it only evaluates the radiopaque material, thus excluding the water under analysis, this is a factor that must be considered because the MTA is a hydrophilic cement. We also used the specimens that simulates the clinical aspect in a better way and allowing to compare the data with other studies that used the retrobuturing cement in cavities in replicates of natural acrylic teeth (Hungaro Duarte *et al.* 2012, Weckwerth *et al.* 2012, Cavenago *et al.* 2014, Canali *et al.* 2016).

High volumetric loss may increase the possibility of bacterial infiltration and consequently lead to failure in the endodontic procedure (Wu *et al.* 1995). The alteration volumetric is a property related to the dissociation and degradation of constituents of the material by action of contact with the surrounding liquid. Thus the larger contact area, as found in samples according to ISO, increases the possibility of greater alteration volumetric. The results showed that the volumetric alteration was greater than 3% for tested materials independent of the contact with blood or not, suggesting greater loss of component. With exception of the CSCT2 the experimental cement which showed a volumetric change when in contact with blood below 3%, suggesting thhe reaction with the blood formed components less succetible to the degradation or dissolution. The CSCT2 after contact considered acceptable because they was below 3%, but the important fact revealed by this study is that the blood did not affect this property, as evaluated in other studies (Bortoluzzi *et al.* 2009, Hungaro Duarte *et al.* 2012, Cavenago *et al.* 2014, Canali *et al.* 2016). Probably the association of 20% Barium sulfate favoured a reaction with the blood which decreased the solubility of the material.

The alkaline pH and ion release are important chemical properties once corroborate with repair process, stimulating the mineralization (Holland et al., 2002). All the cements showed an alkaline pH independent of the evaluated period, this result is in agreement with the specific Literature (Islam *et al.* 2006, Bortoluzzi *et al.* 2009, Parirokh & Torabinejad 2010, Vivan *et al.* 2010, Duarte *et al.* 2012). The groups where

MHP (group VI) was exposed to water or blood showed a higher pH when compared to others, probably due to higher quantity of calcium silicate in the compostion. Calcium silicate react with the water and the one of the product of the hidration is the protlandite which is calcium hydroxide (Duarte *et al.* 2003, Santos *et al.* 2005, Tanomaru-Filho *et al.* 2009). Dissociation of calcium hydroxide results in high pH and detection of high levels of calcium. The higher pH levels occured in the initial periods, before the complete the set of the cements, corroborating with other studies (Duarte *et al.* 2003, Vivan *et al.* 2010). The pH level decreased over time, but remained alcaline, showing that in later periods occurs the hydration of calcium silicate. In relation to the MHP cement, the cements presented a near pH. Regarding the release of calcium ions, the highest values still occurred for MHP. Atomic absorption spectrophotometry with cathode lamp detects calcium in ionized form or not.

In other studies, the results on pH and the release of calcium ions from MTA Angelus are conflicting (Oliveira *et al.* 2010). The MTA Angelus produced a higher pH value and release of calcium ions than GMTA within 168 hours after mixing (Duarte *et al.* 2003). The pH and calcium release were lower in the MTA Angelus than in the MTA (Reyes-Carmona *et al.* 2009). The pH and release of calcium ions between the MTA and the MTA Angelus were not significantly different (Parirokh & Torabinejad 2010). The differences between these studies can be attributed to the measurement time after mixing the material (Chng *et al.* 2005) and to the effect induced by modifying the solution for the incubation of the material (Duarte *et al.* 2003, Islam *et al.* 2006).

Cytotoxicity tests in cell culture are considered initial because they determine in a preliminary way the behavior of the materials under test. In this type of study, cells isolated in culture medium are evaluated in relation to cell death when exposed to a certain material. A number of studies have evaluated the cytotoxicity of MTA and Portland cements, as we did in the present study (Saidon *et al.* 2003, De Deus *et al.* 2005, Hwang *et al.* 2009). Thus, with the importance of the biological properties of endodontic cements, such as the release of nitric oxide, since for tissue repair, both pulp and periapical, it is imperative that the repair materials do not stimulate an exacerbated inflammatory response, that is, do not promote alteration in the inflammatory process (Al-Hiyasat *et al.* 2010, Silva *et al.* 2014). The measurement of NO production by the Griess method (Tanomaru Filho *et al.* 1998), as performed in our study, provides additional data for the analysis of cytotoxicity of materials, as well as

being a well established and widely used procedure used in the studies related to its quantification (Andrade Vitral *et al.* 2008).

The contact of the endodontic cements, either directly or indirectly, with the periapical tissues can stimulate inflammatory cells, mainly macrophages (Brackett *et al.* 2009, Sousa *et al.* 2009). These are involved in the apical healing process and can be activated by various stimuli that are capable of enhancing their biological activities. Their main activity is phagocytosis, and during this process, they release large quantities of mediators that attract the neighboring cells to the affected area in order to reconstruct it (Perassi *et al.* 2004). They have a high capacity for the synthesis and secretion of intra- and extracellular substances, such as NO whinch is a nitrogen intermediates (Ialenti *et al.* 1992, Perassi *et al.* 2004). Macrophages are prevalent in endodontic infections and are the major cells of the immune system that destroy microorganisms. NO is a reactive intermediate species of nitrogen oxide, synthesized by the activity of nitric oxide synthase, an enzyme present in macrophages. Substances generated from the reaction of NO molecules with themselves or with other molecules, such as reactive oxygen species, act on several microorganisms, including *E. faecalis* (Baik *et al.* 2008, Prolo *et al.* 2014, Weiss & Schaible 2015). NO is released when macrophages come into contact with a foreign particle in an attempt to perform phagocytosis. When released in large quantities can increase the cellular metabolism and potentiate the inflammatory process; and in small amounts, favors the process of isolation of the foreign body, cytodifferentiating fibroblasts and exacerbating the protein and collagen synthesis, which characterizes post-obturation repair with formation of fibrous capsule or mineralized tissue (Weiss & Schaible 2015).

The results of our study showed that after 24 hours NO release was decreased between groups. The cytotoxicity of all with NO release was acceptable since the levels of these alterations are so discrete without statistically significant representation, corroborating with other studies that showed MTA less cytotoxic than other materials (Torabinejad *et al.* 1995) and differing from other findings (Scelza *et al.* 2012). However, knowing the importance of the biological behavior of endodontic cements, which should maintain local homeostasis in order to not interfere in the repair and inflammatory processes, it is fundamental that endodontic cements do not promote cell death, keeping the cells immune and inflammatory.

CONCLUSION

It was concluded that the contact with the blood do not interfered in the properties of the materials. The materials presented similar alteration volumetric and citotoxicity in relation to the MTAHP. The MTAHP cement presented the highest pH and calcium ion release. For the experimental cements, the CSCT4 presented the higher calcium release. The CSCT2 in contact with the blood presented the lower volumetric alteration.

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Table 1 - Median (Med), Minimum (Min) and Maximum (Max) values of percentage for solubility immediately after currring (T0) and in seven days (T7) of immersion in blood and distilled water.

Groups	*Immersion Substances*	*T0 Med (Min – Max)*	*T7 Med (Min – Max)*	*Percentage*
CS	**Blood**	$1,86^{a}$ (1,15 - 2,38)	$1,75^{b}$ (0,73 - 2,15)	$7,96^{ABa}$ (0,84 - 36,35)
	DW	$1,87^{a}$ (1,17 - 2,08)	$1,76^{b}$ (1,15 - 1,98)	$6,44^{ABa}$ (1,20 - 10,31)
CSCT1	**Blood**	$1,20^{a}$ (1,20 - 1,92)	$1,23^{a}$ (1,23 - 1,46)	$4,24^{ABa}$ (-8,28 - 23,64)
	DW	$1,77^{a}$ (1,26 - 1,96)	$1,57^{b}$ (1,01 - 1,84)	$6,935^{ABa}$ (4,13 - 23,41)
CSCT2	**Blood**	$1,39^{a}$ (0,98 – 2,17)	$1,33^{b}$ (0,97 - 2,17)	$2,88^{Ba}$ (0,27 - 27,04)
	DW	$1,39^{a}$ (1,27 - 1,79)	$1,14^{b}$ (0,41 - 1,54)	$12,61^{ABa}$ (0,39 - 68,79)
CSCT3	**Blood**	$1,33^{a}$ (1,14 - 2,09)	$1,26^{b}$ (1,07 - 1,99)	$5,17^{ABa}$ (2,56 - 7,05)
	DW	$1,69^{a}$ (0,88 - 2,10)	$1,67^{b}$ (0,81 - 2,04)	$4,45^{ABa}$ (0,90 - 13,89)
CSCT4	**Blood**	$1,72^{a}$ (1,21 - 2,85)	$1,16^{b}$ (0,98 - 1,51)	$28,19^{Aa}$ (17,41 - 57,95)
	DW	$1,31^{a}$ (0,82 - 1,45)	$1,22^{b}$ (0,80 - 1,25)	$9,21^{ABb}$ (2,46 - 15,55)
MHP	**Blood**	$1,32^{a}$ (1,05 - 2,00)	$1,22^{b}$ (1,00 - 1,78)	$7,79^{ABa}$ (0,86 - 17,27)
	DW	$2,27^{a}$ (1,52 - 2,84)	$1,97^{b}$ (1,49 - 2,71)	$3,34^{ABa}$ (0,80 - 24,61)

Different lowercase letters show the statistically significant difference in the percentage in the intra-group comparison (P<0.05). Different Capital letters show statistically significant differences in the percentage of volumetric change in the comparison between the groups.Different lowercase letters show the statistically significant difference between the initial and final volumes in the intra-group comparison (P<0.05).

Table 2 - Median (Med), Minimum (Min) and Maximum (Max) values for pH in the different groups evaluated over time.

Groups	*Immersion Substances*	*3hs Med (Min – Max)*	*24hs Med (Min – Max)*	*72hs Med (Min – Max)*	*168hs Med (Min – Max)*	*360hs Med (Min – Max)*
CS	**Blood**	$7,54^{ABCD}$ (7,42 - 7,57)	$7,62^{AB}$ (7,51 - 7,87)	$7,59^{AB}$ (7,39 - 7,66)	$7,02^{AB}$ (6,77 - 7,41)	$6,60^{BC}$ (6,51 - 6,94)
	DW	$6,93^{ABCD}$ (6,86 - 7,09)	$6,97^{ABC}$ (6,83 - 7,08)	$7,15^{ABC}$ (6,93 - 7,65)	$6,46^{B}$ (5,40 - 6,63)	$6,66^{BC}$ (6,57 - 7,07)
CSCT1	**Blood**	$7,10^{ABCD}$ (6,26 - 7,90)	$7,54^{AB}$ (7,08 - 8,17)	$7,64^{AB}$ (7,53 - 9,33)	$7,03^{A}$ (6,67 - 9,89)	$7,92^{B}$ (6,56 - 9,23)
	DW	$6,75^{CD}$ (6,63 - 6,83)	$6,81^{ABC}$ (6,75 - 6,86)	$6,87^{ABC}$ (6,75 - 6,97)	$6,88^{AB}$ (6,75 - 6,97)	$6,74^{BC}$ (6,64 - 6,80)
CSCT2	**Blood**	$7,56^{ABC}$ (7,32 - 7,81)	$7,59^{AB}$ (7,46 - 7,78)	$7,29^{ABC}$ (6,94 - 7,51)	$6,68^{AB}$ (5,94 - 7,70)	$6,85^{BC}$ (6,67 - 6,99)
	DW	$6,79^{BCD}$ (6,63 - 7,37)	$6,93^{ABC}$ (6,66 - 8,89)	$7,05^{ABC}$ (6,84 - 7,10)	$6,80^{AB}$ (6,49 - 6,89)	$6,45^{C}$ (6,31 - 6,74)
CSCT3	**Blood**	$7,75^{AB}$ (7,49 - 8,06)	$7,63^{AB}$ (7,44 - 7,94)	$7,22^{ABC}$ (6,75 - 9,19)	$6,78^{AB}$ (6,37 - 8,87)	$7,28^{BC}$ (6,68 - 7,41)
	DW	$6,57^{D}$ (6,52 - 6,69)	$6,69^{BC}$ (6,54 - 6,75)	$6,60^{C}$ (6,49 - 6,72)	$6,80^{AB}$ (6,49 - 6,89)	$6,45^{C}$ (6,31 - 6,74)
CSCT4	**Blood**	$7,62^{ABC}$ (7,49 - 8,06)	$7,63^{AB}$ (7,39 - 8,19)	$7,40^{ABC}$ (6,86 - 9,27)	$7,29^{AB}$ (6,65 - 7,82)	$6,76^{BC}$ (6,33 - 6,82)
	DW	$7,17^{ABCD}$ (7,10 - 7,36)	$7,83^{AB}$ (6,96 - 9,25)	$7,41^{ABC}$ (7,33 - 7,69)	$6,57^{AB}$ (5,58 - 6,81)	$6,70^{BC}$ (6,01 - 7,01)
MHP	**Blood**	$7,69^{ABCD}$ (7,54 – 8,76)	$6,38^{C}$ (6,29 - 6,58)	$6,62^{BC}$ (6,35 - 7,52)	$6,58^{AB}$ (6,36 - 6,86)	$6,90^{BC}$ (6,68 - 7,14)
	DW	$7,87^{A}$ (7,59 - 9,82)	$7,82^{A}$ (7,43 - 9,45)	$7,87^{A}$ (7,03 - 8,24)	$7,97^{AB}$ (7,86 - 10,36)	$8,04^{A}$ (7,68 - 9,65)

Different Capital letters show statistically significant differences in the percentage of pH change in the comparison between the groups ($P < 0.05$).

Table 3 - Median (Med), Minimum (Min) and Maximum (Max) values for calcium ion release in the different groups evaluated over time.

Groups	*Immersion Substances*	*3hs Med (Min – Max)*	*24hs Med (Min – Max)*	*72hs Med (Min – Max)*	*168hs Med (Min – Max)*	*360hs Med (Min – Max)*
CS	**Blood**	$1,67^{ABC}$ (0,86- 2,79)	$5,89^{AB}$ (2,75 - 12,27)	$7,67^{AB}$ (2,50 - 10,70)	$5,17^{ABC}$ (1,54 - 7,02)	$6,08^{ABC}$ (3,67 - 8,89)
	DW	$1,01^{ABC}$ (0,25 – 2,88)	$1,46^{AB}$ (0,47 - 4,46)	$2,02^{B}$ (0,16 - 10,11)	$1,33^{C}$ (0,18 - 5,66)	$1,26^{ABC}$ (0,46 - 3,54)
CSCT1	**Blood**	$1,80^{ABC}$ (1,58- 2,87)	$6,72^{AB}$ (2,45 - 15,51)	$9,58^{AB}$ (0,16 - 19,74)	$5,07^{ABC}$ (2,19 - 13,33)	$7,17^{ABC}$ (0,27 - 17,07)
	DW	$0,50^{C}$ (0,26 - 1,34)	$1,88^{AB}$ (0,58 - 2,37)	$1,11^{B}$ (0,38 - 2,87)	$2,81^{BC}$ (1,43 - 4,84)	$0,28^{C}$ (0,0 - 1,11)
CSCT2	**Blood**	$0,82^{BC}$ (0,66- 0,95)	$4,02^{AB}$ (3,47 - 5,79)	$9,39^{AB}$ (6,58 - 11,35)	$7,21^{ABC}$ (4,66 - 14,36)	$7,46^{ABC}$ (5,89 - 9,53)
	DW	$0,52^{C}$ (0,07 - 4,18)	$0,88^{B}$ (0,10 - 5,81)	$0,89^{B}$ (0,35 - 7,91)	$1,40^{BC}$ (0,35 - 3,56)	$0,75^{BC}$ (0,02 - 2,26)
CSCT3	**Blood**	$1,35^{ABC}$ (0,78- 4,17)	$2,42^{AB}$ (1,520 - 6,96)	$7,27^{AB}$ (4,91 - 13,61)	$10,16^{ABC}$ (3,36 - 15,35)	$7,93^{AB}$ (5,28 - 12,93)
	DW	$0,79^{C}$ (0,25 – 1,49)	$1,16^{B}$ (0,02 - 2,60)	$1,20^{B}$ (0,69 - 4,27)	$3,07^{BC}$ (0,87 - 5,20)	$4,91^{ABC}$ (1,63 - 8,39)
CSCT4	**Blood**	$0,82^{ABC}$ (0,37- 4,56)	$2,37^{AB}$ (1,33 - 2,87)	$7,59^{AB}$ (2,86 - 9,86)	$8,97^{AB}$ (8,17 - 13,48)	$7,05^{ABC}$ (1,61 - 10,48)
	DW	$8,82^{AB}$ (1,17 - 12,98)	$7,48^{AB}$ (1,30 - 34,75)	$25,09^{A}$ (14,54 - 30,38)	$5,25^{A}$ (2,39 - 16,49)	$2,51^{ABC}$ (0,15 - 46,26)
MHP	**Blood**	$3,88^{ABC}$ (1,72– 5,79)	$7,24^{A}$ (6,74 - 23,09)	$8,96^{AB}$ (7,06 - 13,01)	$7,69^{ABC}$ (5,35 - 11,79)	$16,30^{A}$ (4,19 - 17,58)
	DW	$15,57^{A}$ (5,59 – 27,18)	$6,62^{AB}$ (1,54 - 11,95)	$6,95^{AB}$ (6,18 - 8,12)	$7,02^{ABC}$ (4,12 - 15,37)	$7,02^{ABC}$ (3,75 - 14,50)

Different Capital letters show statistically significant differences in the release of calcium ions in the comparison between groups ($P < 0.05$).

Table 4 - Median (Med), Minimum (Min) and Maximum (Max) values for the nitric oxide release by the macrofages.

	CSCT1 Median (Min – Max)	***CSCT2 Median (Min – Max)***	***CSCT3 Median (Min – Max)***	***CSCT4 Median (Min – Max)***	***Positive Control Median (Min – Max)***
Nitric Oxide Values	12,41[a] (10,36 - 52,18)	12,19[a] (9,91 - 32,64)	14,00[a] (10,36 - 249,5)	26,96[a] (10,82 - 70,82)	10,82[a] (10,32 - 48,55)

Different lowercase letters show the statistically significant difference between in the intra-group comparison for the nitric oxide ($P<0.05$).

2.3 ARTICLE 3 - Comparison of the physical properties of experimental Calcium Silicate based cement with different radiopacifiers and contac MTA HP when in contact with the blood.

The article presented in this Thesis will be written according to the Journal of Endodontics

ABSTRACT

The aim was to compare the physicochemical experimental Calcium silicate cements containing different radiopacifiers (calcium tungstate, barium sulphate and zirconium oxide) to MTAHP (Angelus) and the interference of the blood in their physical properties (of setting time, radiopacity, flow, thickness, color change and compressive strength). The cements were divided into 6 groups: I – Calcium silicate (CS); II – Calcium Silicate with 30% calcium tungstate and 10% barium sulfate (CSCT1); III – Calcium Silicate with 20% calcium tungstate and 20% barium sulfate (CSCT2); IV – Calcium Silicate with 30% calcium tungstate and 10% zirconium oxide (CSCT3); V – Calcium Silicate with 20% calcium tungstate and 20% zirconium oxide (CSCT4) and VI - MTAHP (MHP). The settling time of the cements was following the ASTM C266/08 standard with 6 specimens, 3 specimens being exposed to the blood. The radiopacity analysis was according to ISO 6876/2001 (ISO, 2001) with 10 specimens, 5 specimens being exposed to the blood. The flow and thickness test performed according to ISO 6876/2001 (ISO, 2001). For the spectrophotometry analysis and using 10 bovine teeth, meaning the INTERNATIONAL COMMISION ON ILLUMINATION (1978) the alteration of the color was analysed. The compression strength was performed according to ISO 1997 (2007) with twelve cylinders being 6 exposed to blood after the total setting time. Resulted in a longer setting time and without interference when exposed to blood, the radiopacity in both groups presented within the norms recommended, the experimental cements had a higher flow and a lower film thickness in comparison to the MTAHP, the spectrophotometric analysis indicated no color change for the cements except for CSCT1 and MTAHP, in the compression strength the experimental cements presented good resistance to compression without interference when in contact with blood. The experimental cements presented good physical properties.

Keywords: MTA. Portland cement. Radiopacifiers.

INTRODUCTION

In mid-1992, in the search for an ideal material to be used in retrograde fillings and perforations, a study was begun by Professor Mahmoud Torabinejad on the material called Mineral Trioxide Aggregate, the MTA, which consists of a compound mainly of calcium oxide. Thus, it was observed that it had higher seal quality than amalgam and Super-EBA and that this seal quality was not compromised when the walls of the cavity were contaminated by the presence of blood. This last observation is particularly important when we intend to use this material in parendodontic surgeries, apitifications, pulp capping, sealing of perforations and pulpotomies (1, 2). The MTA considered the most suitable material to seal the communications between the root canal systems. The MTA is a calcium silicate based cement, composed mainly of Portland cement that is widely used in construction, with the addition of bismuth oxide as radiopacifying agente(3, 4).

The existence of few brands authorized to produce the MTA for clinical use makes the product valued. Numerous studies demonstrating their similarity of the Protland cement in physical, chemical and biological properties to MTA support the use of this materiall as the basis for the development of new calcium silicate based cements (4), with consequent decrease in the final value of the product in comparison to the MTA.

Although the MTA has satisfactory physical, chemical and biological properties, some aspects may limit its use among them the high cost, the low flow and the possibility of staining of the dental structures (5, 6). During its use in parendodontic surgery, pulpotomy, root perforation and in apicification, the MTA contact with the blood and tissue fluids. The blood, in contact with MTA could have some detrimental effect on its physical properties. At the microstructure level, MTA blood contamination may result in the absence of certain types of crystals and chemical components, which may explain the change in their physicochemical properties (7).

The high settling time of the ProRoot MTA is a disadvantage and is due to the slower hydration process of the cement (8). Bismuth oxide negatively affects the setting time of calcium silicate cements (9, 10).

The absence of radiopacity of the Portland cement requires the addition of a radiopacifying agent that allows its clinical use (11). Bismuth oxide, in addition to dental browning, may interfere with the physical-chemical properties of cements, affecting the hydration process (12, 13). There is an increase in porosity and decrease in the

compression forces of cement (14). Therefore, the ideal one would be to find radiopacifiers with better characteristics so as not to interfere in the properties of the cements.

The addition of this radiopacifier to Portland increases the setting time significantly (4, 6, 10, 15). The same is not verified with other radioactifiers such as zirconium oxide (10). Reduced setting time is important to allow the placement of a restorative material in the same session, reducing the number of clinical sessions.

The sandy consistency and the low viscosity are characteristics of the MTA that difficult its insertion and complete sealing (16). Studies have found that the addition of propylene glycol associated with distilled water from the MTA increased prey time and improved flow (16, 17). Portland cement also has a low flow, preventing correct filling in certain cases. The addition of components that promote a greater flow to these cements can be beneficial in the quality of the sealing, in the case of this study we add next to the distilled water the glycerine because it does not have an irritant property equal to the propylene glycol.

Another factor that has been discussed is the potential of MTA to cause dimming of dental structures. This feature is highly undesirable for endodontic materials. The bismuth oxide has been pointed as the element responsible for the darkening verified after the use of the MTA (18, 19). Therefore, alternative radiopacifiers have been studied to replace bismuth oxide (11, 20, 21).

The search for new materials able to meet the needs of those already available in the market is essential for scientific advancement and consequent improvement of endodontic treatment. The objective is to investigate experimental cements based on Portland cement containing different radiopacifiers (calcium tungstate, barium sulfate and zirconium oxide) compared to MTAHP (Angelus) for blood interference by checking for possible changes in its properties (prey time, radiopacity, flow, thickness, color change and compression force), thus seeking better physical and chemical properties.

METHODOLOGY

The materials used in the analyzes were the MTA HP (Angelus, Londrina, Brazil), Itaú structural white Portland cement, glycerine, calcium tungstate, zirconium oxide and barium sulfate. The materials will be divided into 6 (six) groups:

Group I (CS) - Portland Cement + Liquid (70% distilled water and 30% glycerin)

Group II (CSCT1) - 60% Portland cement + 30% Calcium tungstate and 10% barium sulphate + Liquid (70% distilled water and 30% glycerin)

Group III (CSCT2) - 60% Portland cement + 20% Calcium tungstate and 20% barium sulphate + Liquid (70% distilled water and 30% glycerin)

Group IV (CSCT3) - 60% Portland cement + 30% Calcium tungstate and 10% Zirconium oxide + Liquid (70% distilled water and 30% glycerin)

Group V (CSCT4) - 60% Portland cement + 20% Calcium tungstate and 20% Zirconium oxide + Liquid (70% distilled water and 30% glycerin)

Group VI – MTAHP.

For the distributions of the proportions of the experimental mixtures a precision analytical balance was used, just as the blood used in the research was human blood collected in a specific laboratory with the consent of a voluntary donor.

This study was approved by Human Research Ethics Committee of State University of São Paulo (CAAE – 94400518.0.00005417) and was approved by ethics committee on the use of animals (Registry 020/2018).

Setting time analysis

The settling time of the cements was in accordance with ASTM C266 / 08. The spatulated cements were inserted in metal rings of 10mm of internal diameter and 2mm of thickness. Six specimens were used for each cement, three specimens was exposed with 1mL of blood inserted by a needle on top of the samples. The specimens were kept in an oven at 37 ° C ± 1 ° C temperature and 95% ± 5% humidity during the test. After 180 seconds of the start of the spatulation, the specimens were subjected to vertical pressure marking using Gilmore needles. For the initial prey time, the needle used was 113.4 g, followed by the 453.6 g needle for the final prey analysis. Finally, the times, in minutes, elapsed from the beginning of the spatulation to the moment in which it was not possible to visualize the marking of each needle on the surface of the samples, representing the initial and final prey of the cements.

Analysis of radiopacity

For analysis of the radiopacity, it was used 10mm metal rings of internal diameter and 2mm thickness, in accordance with ISO 6876/2001 (ISO, 2001). 10 specimens were prepared for each cement, 5 specimens was exposed with 1mL of

blood inserted by a needle on top of the samples. The spatulated cements were inserted into the rings and kept in an oven at 37 ° C and 100% moisture until complete prey. He radiographed the samples in D occlusal film (Kodak Comp, Rochester, New York, USA) along with an aluminum scale with variations from 2 to 16mm (2mm increments). Manually processed radiographs employing revealing and fixative solution (Kodak, São José dos Campos, São Paulo, Brazil). After processing, he scanned the radiographs and analyzed in the program Digora 1.51 (Soredex, Helsinki, Finland). The determination of the radiopacity in millimeters of aluminum was performed according to (11):

$$\frac{DRM - DRPAA \times 2}{DRPAS - DRPAA} + \text{mm Al step below value} = \text{radiopacity at mm Al}$$

DRM - Radiographic density of material

DRPAA - Radiographic density of the aluminum step below

DRPAS - Radiographic density of the upper aluminum pitch

Analysis of the flow and thickness

The flow and thickness test performed according to ISO 6876/2001 (ISO, 2001). A total volume of 0.5mL of cement will be spatulated and placed in the center of a glass plate. In the flow after 3 minutes of the beginning of the spatulation, another glass plate of mass 20 ± 2g was adapted on the plate containing the cement and on both a weight corresponding to 100g. After 10 minutes the weight was removed and the largest and smallest diameter of the cement were measured using a digital caliper (Mitutoyo MTI Corporation, Tokyo, Japan). Considering the average of the two diameters the flow of the cement and made 3 measurements for each cement variable. For the thickness used 2 plates of glass of 5mm. A total volume of 0.5mL of cement was spatulated and placed in the center of one of the plates and the other glass plate positioned centrally on the previous one. After 3 minutes, a weight corresponding to 150N was placed vertically on the set of plates. After 7 minutes, the weight and thickness of the two plates were removed with the interposed cement measured with a digital caliper (Mitutoyo MTI Corporation). The film thickness was established as the thickness difference between the two plates with and without the interposed cement. Repeated 3 times for each cement.

Color change analysis

For the spectrophotometric analysis we used 10 bovine teeth for each group. For color evaluation with the Vita Easyshade spectrophotometer (Vita-Zanhnfabrik, Germany) we used the criteria by INTERNATIONAL COMMISION ON ILLUMINATION (1978). First the initial color was measured. Then, the coronary opening was performed cleaning the chamber and the filling with the spatulated cements and the sealing with resin. It was analyzed after 7, 15, 30 and 60 days, and for each period 3 analyzes were performed for each tooth. The difference between the initial and final color, or the distance between the two colors on the axes, known as (ΔE) will be calculated using the formula: Δ E = [(Δ L*) 2 + (Δ a*) 2 + (Δ b*) 2] 1/2 (10)]

Analysis of the strength of the compression strength

Performed according to ISO 1997 (2007). Cylindrical molds were produced with 6 millimeters in diameter and 12 millimeters in height with flat ends. Twelve cylinders for each group being 6 exposed with 5mL of blood after the total prey time. In Eppendorfs the cylinders were stored in an incubator at 37 ° C and 100% humidity by seven days. The cylinders were compressed using a universal test machine (Instron 1195, High Wycombe, UK) with a crosshead speed of 1mm / min until a failure was found in accordance with BS EN 9917-1 (British Standard Institution 2003). The maximum load required to fracture the samples was recorded.

Statistical analysis

The results were submitted to D'Agostino and Pearson tests for normal distribution. In cases of absence of normality, the Kruskal-Wallis non-parametric test was used. The tests that had normal use the parametric ANOVA test. For all tests the significance level of 5%

RESULTS

The results of the initial (min), final setting time (Min), radiopacity (mmAl) and the compression strength (mPa) of the evaluated cements shown in Table 1. In relation to the initial setting time, experimental CSCT4 cement presented the highest mean with blood and with distilled water (blood 129.0 ± 8.54 / water 123.0 ± 7.00). MTAHP (blood 18.33 ± 4.50 / water 15.33 ± 3.05) presented the lowest values, with significant difference in relation to the other cements. All other comparisons presented a

statistically significant difference (P<0.05). In the intragroup comparison, there were no significant differences. Regarding the final setting time, the experimental cement CSCT2 (blood 349 ± 24.64 / water 351 ± 13.53) was the one that presented the highest average, being statistically significant in relation to the other cements evaluated. MTAHP (blood 116.7 ± 8.08 / water 107.7 ± 10.21) and CS (blood 132.7 ± 8.50 / water 127.7 ± 1.52) had statistically the lowest mean compared to other cements. All other comparisons presented a statistically significant difference (P<0.05).

In relation the radiopacity, in the group exposed to blood, the highest radiopacity occurred for group CSCT4 (12.47 ± 0,64) did not differentiate with the CSCT3 (11.55 ± 0.62), but these obtained statistical differences with all the other groups evaluated (P<0.05). The CS presented the lowest value (2.40 ± 0.16). The groups in contact with the distilled water showed the highest radiopacity for MTAHP (15.31 ± 0.59), which differed statistically from all other groups (P<0.05). The lowest radiopacity occured in the CS (4,35 ± 0,26). In the intragroup comparisons that obtained statistically significant differences when exposed to blood compared to distilled water in the CS, CSCT2, CSCT3 and MTAHP.

At the analysis of the compression strength, the highest values when exposed to the blood occured in the CSCT4 group (14.77 ± 2.35) and the lowest was for the CS group (5.89 ± 1.62) and it presented a statistically significant difference (P<0.05) in releation the other groups. In contact with the distilled water, the greatest force of compression was found for the CSCT3 group (14.90 ± 1.11), obtaining a statistically significant difference (P<0.05) with the CSCT1 group (10.28 ± 1.27) and this one also presented significant differences (P<0.05) in relation the CSCT4 group. The CS group presented the lowest compression strength (6.01 ± 1.60), which obtained statistically significant differences (P<0.05) in comparison with the other groups. Finally, in the intragroup comparison, only CSCT1 showed statistically significant differences (P<0.05) between the exposed to blood and the exposed to distilled water.

The results and statistically significant differences in the flow and thickness of the evaluated cements shown in table 2. For the flow, the CSCT4 group (14.97 ± 1.37) presented the highest result with a statistically significant difference (P<0.05) in relation to the MTAHP group that presented the lowest value (10.19 ± 0.65). The other groups did not present significant differences (P<0.05) between them. As to thickness, CS presented the highest thickness value (1.53 ± 0.16) with a statistically significant difference (P<0.05) in relation to the other cements. The CSCT2 group presented the

lowest result (0.35 ± 0.07), but none of the other groups presented significant differences between them (P>0.05).

The table 3 contains the results and statistical differences of the color change. In relation to 7 day period, the greatest change was for the CSCT1 group (196,1) and was the only that obtained a statistically significant difference (P<0.05) in relation the CSCT4 group (31.75) which presented a minor change of color. At the 15 day period the highest representativeness occurred for CSCT1 (95.64), which presented a statistically significant difference with the CSCT4 group (17.29) which presented the lowest color change and presented statistical significant difference (P<0.05) in relation the CSCT3 and MTAHP groups. For the 30 day period, the greatest change was presented for the MTAHP group (83.35) with a statistically significant difference (P<0.05) in relation the CSCT4 group (12.52) which presented the lowest change.I n relation to 60 day period, the greatest change was presented for the MTAHP group (46.86) which presented statistically significant difference (P<0.05) in relation the CSCT4 group (12.21) which obtained the lowest change. Regarding the intragroup comparison the differences are demonstrated in the table 3.

DISCUSSION

The purpose of this study was to evaluate the physical properties (setting time, radiopacity, flow, thickness, color change and compressive strength) of experimental Portland cements containing different radiopacifiers (calcium tungstate, barium sulfate and zirconium oxide) comparing with the MTAHP, and finally also analyzing the interference of blood in the influence of the some properties of the cements.

In relation to the setting time, related to the hydration of the cement, it is recommended that the cements should not exceed 10% of the specified by the manufacturer, considering that for the experimental cements, it is not possible to adopt this instruction, being necessary the comparison with materials with an approximate composition already available on the Market (6) in and comparing with a comercial which is the MTAHP. The MTA Angelus has an initial and final setting time of around 12 and 48 minutes (22) smaller setting time is explained by the absence of calcium sulphate. Portland cement presents a longer setting time on average between 38-40 minutes for initial setting time and between 135-190 minutes for final setting time (4, 23). The presence of calcium sulphate hinders the reaction of the tricalcium aluminate

to produce tricalcium aluminate hydrate (23). Another aspect that can change the setting time is the powder / liquid ratio. When more liquid is added in the handling, a delay in the cement holding time occurs (24). In agreement with the above the results in relation to the initial setting time, the highest mean was for experimental CSCT4 cement with both blood and distilled water (blood 129.0 ± 8.54 / water 123.0 ± 7.00). MTAHP (blood 18.33 ± 4.50 / water 15.33 ± 3.05) presented the lowest values, with significant difference in relation to the other cements. Regarding the final setting time, the experimental cement CSCT2 (blood 349 ± 24.64 / water 351 ± 13.53) was the one that presented the highest average, being statistically significant in relation to the other cements evaluated. MTAHP (blood 116.7 ± 8.08 / water 107.7 ± 10.21) and CS (blood 132.7 ± 8.50 / water 127.7 ± 1.52) had statistically the lowest mean compared to other cements. Cements based on calcium silicate considered as hydraulic cements. Zirconium oxide and calcium tungstate do not participate in the cement hydration process (21). The availability of smaller amounts of calcium silicate cement resulting from the addition of larger amounts of radiopacifiers results in an increase in the time required for the setting of the material. On the contrary, in cements in which there is a smaller amount of radiopacifier, the proportion of calcium silicate cement will be higher to react with the liquid, resulting in a faster setting time (25). Besides that, the addition of glycerin to the liquid may have interfered with the setting of the cements, as was demonstrated when adding propylene glycol, increasing them in the liquid decreases the volume of water available to react with calcium silicate (16).

The radiopacity recommended by the ANSI / ADA standard is above 3.0 mm Al. The addition of different radiopacifiers has been proposed to provide radiopacity to Portland and thus allow its use (11, 26, 27). According to the MTA, the proportion of bismuth oxide present in its composition is 20% (1), verified a radiopacity equivalent to 7,17 mmAl(28), other studies reported a radiopacity around 6-8 mm Al (4, 27). These findings corroborate with this study when MTAHP in contact with blood (5,87 ± 0,81). Portland's radiopacity ranges from 0.86-2 mm Al (4, 11, 26). Different proportions and different radiopacifiers have been evaluated in Portland from 10, 15, 20, 30 and 50% with variable results, however in all the studies it is possible to observe that the proportion of the radiopacifier has a relation directly proportional to the radiopacity verified for the cements. Thus, the increase in the proportion of radiopacifiers increases the radiopacity, which is expected to occur as in this study where the degree of radiopacity increased due to the proportions of each group of radiopacifiers. In the

group exposed to blood, the highest radiopacity occurred for the CSCT4 group, not differentiating with the CSCT3, but these obtained statistical differences with all the other groups, being CS the lowest value found, which has not been accreted with radiopacifiers, the presence of steel in the blood may have been the cause of the change in radiopacity. The group with distilled water which presented a higher radiopacity was for the MTAHP, differing statistically with all the other groups and CS presenting the lowest radiopacity, and due to the metal rings used for the analysis that was 2mm thick which caused a change due to the increase of material and consequently a higher radiopacity. In comparison intragroup the groups that obtained statistically significant differences when exposed to blood compared to distilled water were CS, CSCT2, CSCT3 and CS. The method of weight proportionation used to add the radiopacifiers of the cements can cause differences, this may explain the differences found in the marks and experimental cements, which may increase the degree of radiopacificity that was used in this study as well. The weight ratio results in a smaller volume of the higher molecular weight substance. The higher molecular weight substance needs a smaller volume to achieve the same weight as a lower molecular weight substance, this occurs with the bismuth oxide present in the MTA (11).

The flow and thickness properties were evaluated to verify the difference between the experimental cements handled with the association of distilled water and glycerine with the MTAHP. It is recommended by ANSI / ADA a flow greater than 20mm and a thickness less than 50μm. The sandy consistency and the low viscosity are characteristics of the MTA that hinder its insertion and complete sealing (16). Portland cement also has a low flow, difficulting the correct filling. The addition of components that promote a greater flow to these cements can be beneficial in the quality of the sealing, in the case of this study we add the 20% glycerine in the liquid in substitution the propylene glycol (16, 17). The results showed that the experimental cements had a higher flow and a lower film thickness compared to the MTAHP which the polymer in the liquid is the Polivynilpirrolidone. Although there has been an improvement in such properties in relation to the MTAHP, they were not in accordance with the recommendations of standard ANSI/ADA No. 57, however, values are recommended by the standard for root filling material where high fluidity and low film thickness are essential for proper distribution of cement in the root canal system (29). The high film thickness may be related to the particle size of Portland cement and radiopacifiers.

Color change in tooth structure is an undesirable consequence of some chemical reactions observed for certain materials when in contact with dental structures (5, 19, 30). The color change after, the application of MTA in contact with the dental surface has been reported (31-33). Factors associated with its darkening have been evaluated and some hypotheses suggested. Most theories suggest that the presence of bismuth oxide would be associated with the color change of the MTA (34). In the study, bovine teeth were used to evaluate the tooth color change after contact with the cements, as well as in other studies (19). Extracted human teeth usually have restorations or cavities that interfere with color analysis. On the other hand, bovine teeth are more easily obtained and have a wide flat surface for an adequate measurement of color, allowing a better standardization of the samples. The dentin of the human and bovine teeth is similar in number of tubules per mm^2 and diameter of the tubules. The spectrophotometer analysis was selected to study the color of the cements. Previous studies have used a similar methodology to evaluate color change of calcium silicate cements (34, 35). In the study, the spectrophotometric analysis indicated no significant color change for the experimental cements, except for the experimental group CSCT1 with higher values in the periods of 7 and 15 days and the group MTAHP in the periods of 30 and 60 days, with formation of gray areas near the interface between cement and dentin. The results for the MTAHP group corroborate with those reported in the Literature (5, 30). Possibly the high presence of Calcium tungstate can favor a darkness.

Compressive strength testing of dental cements is usually performed according to British Standard Institution (2003). In a previous paper testing various proprietary brands, it was concluded that compressive strength testing depends on specific test conditions. The cements were not susceptible to changes in the compressive strength testing procedure at 1 and 7 days, but at 28 days all the fast setting cements had a significantly higher strength with a particular testing mode. Abnormal failure occurred when testing according to British Standard Institution (2003) and the standard deviations for the tests carried out were large. Compressive strength testing of prototype dental cements was thus performed according to British Standard Institution (2005a), which differs from this study that was performed as suggested by the standard ISO 1997-1 (2007). Properties can be altered with the addition of bismuth oxide, the microstructure of the cement can be altered, and the MTA has a weaker microstructure than Portland without bismuth oxide (13). This change in microstructure may be the

factor responsible for the reduction in resistance to compressive strength (14, 26). High concentrations of radiopacifiers may adversely affect compressive strength properties when added to Portland cement (14). Contamination of MTA by blood may result in the absence of certain types of crystals, which may also explain the reduction in resistance to compression Strength (7) the more the MTA is incorporated by blood, the lower the compressive strength of the material (36). The results of the study showed that the experimental cements presented good resistance to compression strength, noting that the greatest compression force exposed to the blood presented was for the experimental group CSCT4 and the smaller one was for the experimental group CS and thus the only ones that presented a statistically significant difference between the groups, showing that the presence of the radiopacifier studied favor the compression strength. In contact with the distilled water the greatest compression strength was found for the CSCT3 group, obtaining a statistically significant difference with the CSCT1 group, and this one presenting significant differences also with the CSCT4 group. The CS group showed the lowest compression force, which obtained statistically significant differences with all the other groups. Finally, in the intragroup comparison only CSCT1 showed statistically significant differences between the exposed to blood and the exposed to distilled water. Cements produced by addition of calcium aluminate cement to Portland cement have a reduced compressive Strength (37). The compressive strength of the cement can be improved by reduction in the water to cement ratio. This is achieved through the use of superplasticing admixtures. The plasticizing action of water reducers is related to their adsorption and dispersing effects in the cement–water system (13).

CONCLUSION

Experimental cements with Portland cement with different radiopacifiers compared to the MTAHP presented longer setting time without blood interference. Radiopacity in both groups was above the standards recommended. The experimental cements had a higher flow and a lower film thickness compared to the MTAHP. The spectrophotometric analysis indicated absence of color change for the cements except for CSCT1 and MTAHP. Finally, in the compression force the experimental cements showed good resistance to compression force without interference when in contact with blood.

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Table 1- Mean (X) and standartdesviation (SD) values for the initial (TI) and final (TF) setting time, radiopacity and compression strength for the simples immersed in blood and distilled water.

		Setting Time		Radiopacity	CompressionStrength
Groups	**ImmersionSubstances**	**TI X ± SD**	**TF X ± SD**	**X ± SD**	**X ± SD**
G1	**Blood**	53,00 ± 2,00^{Da}	132,7 ± 8,50^{Ea}	2,40 ± 0,16^{Ae}	5,89 ± 1,62^{Aa}
	DW	43,00 ± 2,00^{Db}	127,7 ± 1,52^{Ea}	4,35 ± 0,26^{Be}	6,01 ± 1,60^{Ac}
G2	**Blood**	63,67 ± 11,93^{Ca}	195,3 ± 3,05^{Da}	7,35 ± 0,62^{Ac}	14,68 ± 2,70^{Ab}
	DW	44,67 ± 5,13^{Da}	188,7 ± 7,76^{Da}	7,29 ± 0,79^{Ad}	10,28 ± 1,27^{Bb}
G3	**Blood**	98,00 ± 2,00^{Ba}	349 ± 24,64^{Aa}	9,14 ± 0,40^{Ab}	13,66 ± 1,35^{Ab}
	DW	93,67 ± 3,05^{Ba}	351 ± 13,53^{Aa}	13,22 ± 0,62^{Bab}	12,45 ± 1,52^{Aab}
G4	**Blood**	93,67 ± 2,51^{Ba}	300,3 ± 2,08^{Ba}	11,55 ± 0,62^{Aa}	13,26 ± 2,73^{Ab}
	DW	91,00 ± 3,46^{Ba}	300,7 ± 2,30^{Ba}	12,89 ± 0,57^{Bab}	14,90 ± 1,11^{Aa}
G5	**Blood**	129,0 ± 8,54^{Aa}	257,7 ± 3,05^{Ca}	12,47 ± 0,64^{Aa}	14,77 ± 2,35^{Ab}
	DW	123,0 ± 7,00^{Aa}	258,7 ± 2,51^{Ca}	12,65 ± 0,64^{Ab}	14,26 ± 2,59^{Aa}
G6	**Blood**	18,33 ± 4,50^{Ea}	116,7 ± 8,08^{Ea}	5,87 ± 0,81^{Ad}	13,80 ± 1,16^{Ab}
	DW	15,33 ± 3,05^{Ea}	107,7 ± 10,21^{Ea}	15,31 ± 0,59^{Bc}	12,77 ± 1,70^{Aab}

Different lowercase letters show the statistically significant difference between the groupswith blood and DW in the intra-group comparison ($P<0.05$). Different Capital letters show statistically significant differences in the percentage in the comparison between the groups.

Table 2- Mean (X) and standartdesviation (SD) values for the flow and thickness in the different groups release the cements.

	Flow X ± SD	**Thickness X ± SD**
CS	10,47 ± 1,06^{AB}	1,53 ± 0,16^{A}
CSCT1	12,39 ± 1,73^{AB}	0,49 ± 0,19^{B}
CSCT2	12,47 ± 0,44^{AB}	0,35 ± 0,07^{B}
CSCT3	14,14 ± 3,15^{AB}	0,52 ± 0,11^{B}
CSCT4	14,97 ± 1,37^{A}	0,63 ± 0,15^{B}
MTAHP	10,19 ± 0,65^{B}	0,40 ± 0,06^{B}

Different Capital letters show statistically significant differences in the percentage of flow and thickness in the comparison between the groups.

Table 3 - Median (Med), Minimum (Min) and Maximum (Max) values for the discoloration change in the different groups evaluated over time.

	DiscolorationChange			
Groups	**7days Med (Min – Max)**	**15days Med (Min – Max)**	**30 days Med (Min – Max)**	**60days Med (Min – Max)**
CS	66,70^{ABa} (15,77 - 207,1)	34,25^{ABa} (11,93 - 224,1)	41,52^{ABa} (5,04 - 268,8)	24,64^{Aba} (7,75 - 250,1)
CSCT1	196,1^{Aa} (25,85 - 341,6)	95,64^{Aa} (5,20 - 141,7)	52,72^{ABb} (9,82 - 123,9)	40,06^{Abc} (4,10 - 107,9)
CSCT2	74,12^{Aba} (18,75 – 197,0)	49,22^{ABa} (12,07 – 191,7)	39,63^{ABa} (8,18 – 146,1)	35,86^{Aba} (4,78 – 130,7)
CSCT3	43,22^{Aba} (14,41 - 232,0)	79,38^{Aa} (13,15 - 424,5)	46,22^{ABa} (7,43 - 402,0)	30,79^{ABb} (3,63 – 432,2)
CSCT4	31,75^{Ba} (9,00 - 175,4)	17,29^{Ba} (2,58 - 96,59)	12,52^{Ba} (1,12 - 70,38)	12,21^{Ba} (1,57 - 56,21)
MTAHP	69,45^{Aba} (27,64 – 113,5)	63,81^{Aa} (29,25 - 261,6)	83,35^{Aa} (20,58 - 258,3)	46,86^{Aa} (21,01 - 132,2)

Different lowercase letters show the statistically significant difference between in the intra-group comparison ($P<0.05$). Different Capital letters show statistically significant differences in the percentage of discoloration change in the comparison between the groups.

3 DISCUSSION

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The properties of calcium silicate cements have often been evaluated. One of the most important aspects observed is the influence of components added to Portland cement and MTA on the physical, chemical and biological behavior of these materials.

MTA is a powdered material composed of fine hydrophilic particles (SAGHIRI et al., 2011), whose main components are tricalcium silicate, dicalcium silicate, tricalcium aluminate, tricalcium oxide and silicate oxide, originating from Portland cement (FORMOSA, MALLIA, CAMILLERI, 2012) that, in contact with water, produces an exothermic chemical reaction of crystallization of hydrated prohydes, thus gaining mechanical resistance. There are also small amounts of oxides of other minerals that attach physical and chemical properties to the aggregate, especially bismuth oxide that attributes radiopacity. There is a chemical similarity between the MTA and a calcium silicate cement, the Portland cement, used in the construction industry (ESTRELA, et al., 2000). Studies have shown similarity in the constitution of both, differing only by the presence of bismuth oxide in the MTA (CAMILLERI, et al., 2005). As for the physical properties, they presented similar results (ISLAM, CHNG, YAP, 2006) and also biological (SAIDON et al., 2003; DE DEUS et al., 2005). Some characteristics, despite having good physical, chemical and biological properties, may limit the use of MTA, such as high cost, low flow and possibility of staining of dental structures (BELOBROV; PARASHOS, 2011; DUARTE et al., 2012). The search for new materials with capabilities to meet the requirements of those already available in the market, is essential for scientific advancement and, consequently, for improvements in endodontic treatment. Thus, the aim of the experimental cement based on Portland cement was to try to achieve better properties regarding radiopacity, setting time, film flow and thickness, pH, volumetric change, calcium ion release, cytotoxicity with the release of nitric oxide when in contact with macrophages, dental discoloration and compressive strength compared to MTAHP also evaluating the effect when they come into contact with blood.

Alternative radiopacifiers were evaluated in association with Portland cement due to the disadvantages associated with bismuth oxide. The presence of the bismuth

oxide radiopacifier in the MTA composition interferes with the hydration process (CAMILLERI, 2007) and changes the mechanical properties of the cement (COOMARASWAY; LUMLEY; HOFMANN, 2007). In addition, the interaction between bismuth oxide and sodium hypochlorite results in the formation of a black colored precipitate that can change the color of dental structures (CAMILLERI, 2014). The radiopacifier zirconium oxide has been evaluated as an alternative because it shows adequate radiopacity (DUARTE, et al., 2009; CAMILLERI, SORRENTINO, DAMDOT, 2013), does not interfere in the Portland cement hydration reaction, hydroxyapatite deposition and the release of calcium ions (CAMILLERI, CUTAJAR, MALLIA, 2011b; BORGES, et al., 2012; VITTI, et al., 2013). However, calcium tungstate has also been evaluated as an alternative and presents, when associated with Portland, physical and chemical properties characterized by low solubility, high pH and release of calcium ions (Duarte et al. 2012).

The MTA has biological properties that favor tissue repair, being therefore used in different procedures, such as apitifications, pulp capping, sealing of perforations and in apical surgeries (LEE; MONSEF; TORABINEJAD 1993; CHONG; PITT FORD; HUDSON, 2003; HILTON, FERRACANE, MANCL, 2013). This cement presents bioactive properties that include tissue repair and mineralization, induced by the release of calcium ions (HOLLAND et al 2001c; DUARTE et al., 2003; MAROTO et al., 2005; DREGER et al., 2012). These properties are desirable primarily because of direct contact between the material and the pulp or periodontal tissues. During clinical use, contact of the MTA occurs with blood and tissue fluids. The blood, in contact with the obturator material, is incorporated, which could have some detrimental effect on its physical, chemical and biological properties of the obturator material (NEKOOFAR, STONE, DUMMER, 2010; SHAKOUIE et al., 2012, CANALI et al., 2016).

The volumetric change of root end and perforation sealing materials is an important property, since dissolution of the material can allow infiltration, compromising the success of the treatment. In vitro tests evaluating the properties of solubility or volumetric change have used the ANSI / ADA n57 or ISO 6876 specifications, which are based on the difference between the weights before and after the placement of the cement in distilled water (TORABINEJAD et al., 1995; DUARTE et al., 2012; VIVAN et al., 2010; FRIDLAND M, ROSADO R, 2005; TANOMARU-FILHO et al., 2017). However, hydrophilic material can absorb water and increase in weight without

adequately demonstrating its solubility. MTA has low or almost no solubility, which is attributable to the addition of bismuth oxide (RAO, et al., 2009; PARIROKH; TORABINEJAD, 2010). High solubility may increase the possibility of bacterial infiltration and, consequently, lead to failure in endodontic treatment (WU; WESSELINK; BOERSMA, 1995). Solubility is a property directly related to the dissociation of the constituents of the material by action of contact with the surrounding liquid.

The alkaline pH and release calcium ions, which are important chemical properties, help repair by stimulating the process of tissue mineralization (HOLLAND et al., 2002; CANALI et al., 2016, PARIROKH et al., 2010, VIVAN et al., 2010, DUARTE et al., 2012, BORTOLUZZI et al., 2009, ISLAM et al., 2006). Calcium silicate cements are known to have pH values and release of high calcium ions (DUARTE et al., 2003; SANTOS, et al., 2005; TANOMARU-FILHO, et al., 2009). Such cements are rich in calcium oxide, which, in contact with tissue fluids or water, are converted to calcium hydroxide (HOLLAND et al., 1999). Dissociation of calcium hydroxide results in high pH and detection of high levels of calcium. The release of calcium hydroxide in the form of Ca^{+2} ions and hydroxyl OH^{-}, results in alcaline pH (SANTOS et al., 2005; CAMILLERI, 2007, 2008c).

Several studies have evaluated the cytotoxicity of MTA and Portland cements (SAIDON et al., 2003; DE DEUS et al., 2005; HWANG et al., 2009) as we did in this study. Thus, with the importance of the biological properties of endodontic cements, such as the release of nitric oxide, since, for tissue repair, both pulp and periapical, it is imperative that the obturator materials do not stimulate an exacerbated inflammatory response, do not promote change in the inflammatory process (AL-HIYASAT; TAYYAR; DARMANI, 2010; SILVA, 2014).

In relation to the setting time, related to the hydration of the cement, it is recommended that the cements should not exceed 10% of the specified by the manufacturer, considering that for the experimental cements, it is not possible to adopt this instruction, being necessary the comparison with materials with an approximate composition already available on the market (DUARTE et al., 2012a) in this study compared to the MTAHP (Angelus). The MTA Angelus has an initial and final setting time of around 12 and 48 minutes (BORTOLUZZI et al., 2009) smaller setting time

explained by the absence of calcium sulphate. Portland cement presents a longer setting time on average between 38-40 minutes for initial and between 135-190 minutes for final (CAMILLERI, 2008b; ISLAM, CHNG, YAP, 2006). The presence of calcium sulfate hinders the reaction of tricalcium aluminate to produce tricalcium aluminate hydrate (CAMILLERI, 2008b). Another aspect that can change the setting time is the powder / liquid ratio. When more liquid added in the manipulation, there is a delay in the cement setting time (CAVENAGO et al., 2014).

The radiopacity recommended by the ANSI / ADA standard is above 3.0 mm Al. The addition of different radiopacifiers has been proposed to provide radiopacity to Portland and thus allow its use (DUARTE et al., 2009; SALIBA et al., 2009; CAMILLERI 2010a; CAMILLERI, GANDOLFI 2010b; CAMILLERI, CUTAJAR, MALIA, 2011). According to the MTA, the proportion of bismuth oxide present in its composition is 20% (TORABINEJAD et al., 1995), a radiopacity equivalent to 7,17 mm Al (TORABINEJAD et al., 1995a), others studies reported a radiopacity around 6-8mm Al (ISLAM, CHNG, YAP, 2006; CAMILLERI GANDOLFI, 2010b). Portland's radiopacity ranges from 0.86-2 mm Al (ISLAM, CHNG, YAP, 2006; DUARTE et al., 2009; SALIBA et al., 2009). Thus, the addition of a radiopacifier is required. Different proportions and different radiopacifiers have been evaluated in Portland from 10, 15, 20, 30 and 50% with variable results, however in all the studies it is possible to observe that the proportion of the radiopacifier has a relation directly proportional to the radiopacity verified for the cements.

ANSI/ADA a flow greater than 20mm and a thickness less than 50μm recommend it. The sandy consistency and the low viscosity are characteristics of the MTA that hinder its insertion and complete sealing (DUARTE et al., 2012b). Portland cement also has a low flow, preventing correct filling in certain cases. The addition of components that promote a greater flow to these cements can be beneficial in the quality of the sealing, in the case of this study we add next to the distilled water the glycerine because it does not have an irritant property equal to the propylene glycol (DUARTE et al., 2012b; HOLLAND et al., 2007).

Color change in tooth structure is an undesirable consequence of some chemical reactions observed for certain materials when in contact with dental structures (JACOBOVITZ, DE PONTES LIMA, 2009; BELOBROV, PARASHOS, 2011;

LENHERR et al., 2012). The color change after, the application of MTA in contact with the dental surface has been reported (MARCIANO et al., 2014; FELMAN, PARASHOS, 2013; GUIMARÃES et al, 2015). Factors associated with its darkening have been evaluated and some hypotheses suggested. Most theories suggest that the presence of bismuth oxide would be associated with the color change of the MTA (CAMILLERI, 2014; VALLES et al., 2013b).

Properties with the addition of bismuth oxide, the microstructure of the cement can be altered, and the MTA has a weaker microstructure than Portland without bismuth oxide (CAMILLERI, 2007). This change in microstructure may be the factor responsible for the reduction in resistance to compressive force (COOMARASWAMY, LUMLEY, HOFMANN, 2007; SALIBA et al., 2009). High concentrations of radiopacifiers may adversely affect compressive strength properties when added to Portland cement (COOMARASWAMY, LUMLEY, HOFMANN, 2007). Contamination of MTA by blood may result in the absence of certain types of crystals, which may also explain the reduction in resistance to compression force (NEKOOFAR, STONE e DUMMER, 2010) the more the MTA is incorporated by blood, the lower the compressive strength of the material (NEKOOFAR, OLOOMI, SHEYKHREZAE et al, 2010; CANALI et al.,2016).

4 CONCLUSIONS

4 CONCLUSIONS

According to the results obtained with the experiments was possible to concluded that:

- The alteration volumetric is influenced according to the moment of immersion of the specimen in the water;
- The use of different radiopacifiers and glicerin in combination with Portland cement compared to the MTAHP resulted in a radiopacity in all groups within the recommended standard;
- The use of different radiopacifiers and glicerin in combination with Portland cement favoured a longer setting time and without interference when exposed to blood;
- The experimental cements exhibited a higher flow and lower film thickness compared to the MTAHP;
- The experimental cements exhibited a similar volumetric alteration in relation the MTAHP and the immersion in blood interfered at this alterations;
- The pH and calcium release were slightly higher for the MTAHP cement, but all groups were very similar;
- The cytotoxicity of all with NO release was acceptable since the levels of these changes are so discrete;
- The spectrophotometric analysis indicated absence of color change for the cements, except for CSCT1 and MTAHP, which presented a slight significant color change;
- At the compression force, the experimental cements presented good resistance without interference when in contact with blood.

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APPENDIXES

APENDIX A - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article **Effect of the immersion moment at the volumetric alteration of the Mineral Trioxide Aggregate HP** will be included in the Thesis of the student (Lyz Cristina FurquimCanali) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, June 16th 2019.

Lyz Cristina Furquim Canali
Author Signature

Rafaela Fernandes Zancan
Author Signature

Rodrigo Ricci Vivan
Author Signature

Mario Tanomaru-Filho
Author Signature

Marco AntonioHungaro Duarte

Author Signature

ANNEXES

ANNEX A – Ethics committee approval

USP - FACULDADE DE ODONTOLOGIA DE BAURU DA USP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito do sangue nas propriedades físico-químicas e escurecimento de cimento de silicato experimental e do MTA HP

Pesquisador: Lyz Canali

Área Temática:

Versão: 3

CAAE: 94400518.0.0000.5417

Instituição Proponente: Universidade de Sao Paulo

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.055.154

Apresentação do Projeto:

Trata-se de um projeto de doutorado intitulado "Efeito do sangue nas propriedades físico-químicas e escurecimento de cimento de silicato experimental e do MTA HP", tendo como responsável principal a pesquisadora Lyz Cristina Furquim Canali e equipe de pesquisa composta pelo professor e orientador Dr. Marco Antonio Hungaro Duarte. O objetivo deste estudo será analisar um cimento experimental (CE) à base de cimento Portland, contendo diferentes concentrações dos agentes radiopacificadores tungstato de cálcio, sulfato de bário e óxido de zircônio e o MTA HP quanto às propriedades físico-químicas (radiopacidade, tempo de presa, espessura de filme, escoamento, solubilidade, resistência à compressão e avaliação da descoloração dental, pH e liberação de íons cálcio) quando entram em contato com o sangue ou saliva artificial ou água deionizada. Os cimentos serão divididos em 6 grupos:

I – Cimento Portland;

II – CE com 30% de tungstato de cálcio e 10% de sulfato de bário;

III – CE com 20% de tungstato de cálcio e 20% de sulfato de bário;

IV – CE com 30% de óxido de zircônio e 10% de sulfato de bário;

V – CE com 20% de óxido de zircônio e 20% de sulfato de bário;

VI – MTA HP.

Para o preparo dos cimentos experimentais será adicionado glicerina à água destilada na proporção de 30% em volume. Para os testes de radiopacidade, espessura de filme, escoamento

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Continuação do Parecer: 3.055.154

serão seguidas as especificações ISO 6876/2001. Na determinação do tempo de presa será empregada a norma ASTM C266/2008. Para determinação do pH, solubilidade e liberação de íons cálcio serão utilizados dentes de acrílico com cavidades retrógradas preenchidas com os cimentos colocados em contato com o sangue e imersos em água MilliQ. A solubilidade será analisada volumetricamente em Micro CT. As análises serão realizadas após os períodos de 3, 7 e 15 dias. A resistência à compressão através da norma ISO 9917/2007. Para avaliação da descoloração dental serão utilizados 10 dentes bovinos para cada grupo (60 dentes). Os dentes terão as câmaras pulpares limpas e obturadas com os cimentos. Após os períodos de 7, 15, 30 e 60 dias os dentes serão submetidos à espectrofotometria. Os resultados serão submetidos a teste de normalidade de D'Agostino e Pearson para posterior escolha do teste adequado para cada análise, com nível de significância de 5%. Os pesquisadores necessitarão de um participante para doação de sangue. Toda pesquisa deve acontecer nas dependências do departamento de Dentística, Endodontia e Materiais Odontológicos, disciplina de Endodontia da FOB/USP.

Objetivo da Pesquisa:

Hipótese:

As hipóteses nulas a serem testadas são de que o cimento obturador à base de cimento Portland e o MTA não sofrerão interferências nas propriedades de tempo de presa, solubilidade, resistência à compressão, descoloração dental, pH e liberação de íons cálcio quando em contato com o sangue.

Objetivo Primário:

O objetivo do estudo será analisar um cimento experimental à base de cimento Portland, contendo diferentes concentrações dos agentes radiopacificadores tungstato de cálcio, sulfato de bário ou óxido de zircônio, com 30% de glicerina em volume no líquido e o MTA HP quanto às propriedades físicas (radiopacidade, tempo de presa, espessura de filme, escoamento, solubilidade, resistência à compressão e avaliação da descoloração dental), químicas (pH e liberação de íons cálcio).

Objetivo Secundário:

Analisar se o cimento Portland com maiores concentrações dos agentes radiopacificadores aumentarão a radiopacidade, porém sem interferir nas propriedades tempo de presa, escoamento, espessura de filme, solubilidade, resistência à compressão, pH e liberação de íons cálcio. Adicionalmente, será testada a hipótese de que o tungstato de cálcio adicionados ao cimento Portland não causarão o efeito de descoloração dental como já foi observado e publicado do MTA Angelus, com e sem contato com o sangue.

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Avaliação dos Riscos e Benefícios:

Riscos:

O risco será na coleta de sangue do voluntário onde a auxiliar de enfermagem pode errar o procedimento, sendo o voluntário esclarecido no TCLE sobre os riscos, mas não há danos sobre a saúde física ou mental ou qualquer outro tipo de problema que impeça de ser realizado. Riscos que pode ocorrer durante a coleta através da enfermeira são: Usar agulhas de calibre muito fino ou muito grosso; aspirar o sangue violentamente após atingir a veia; produzir estase venosa prolongada pelo uso do garrote; erro na direção da agulha; transfixação da veia; formação de edema após a punção por extravasamento de sangue sob pele. A pesquisa na execução de sua metodologia fornece riscos de erro de execução nos espécimes como preparação do cimento com a manipulação fora do padrão, erro na coleta de informações quando estiver nas máquinas, colocar algum dado errado ou algum espécime estiver fora do padrão com bolha ou irregular e não tiver sido excluído, ou ainda na coleta de dados trocar ordem ou números. Mas será uma pesquisa bem controlada e inspecionada e em ambiente laboratorial.

Benefícios:

Serão as respostas das interferências das propriedades dos cimentos experimentais e do MTA desses materiais obturadores quando em contato com sangue comparado com os grupos controles, para obtermos uma certeza de sua possibilidade de interferência de suas propriedades físico e químicas quando há esse contato do material por esses agentes em uma situação clínica na endodontia, no caso de obturação retrógrada ou de cirurgia parendodôntica, casos que ocorrem na endodontia.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa bem interessante na qual com os resultados que se espera obter poderá se verificar dos materiais obturadores observados quais as interferências das propriedades dos cimentos experimentais e do MTA quando em contato com sangue comparado com os grupos controles. Na pesquisa em si não existem práticas invasivas (exceção seria a coleta de sangue) ou qualquer problema que torne a pesquisa inviável do ponto de vista ético, uma vez apresentados todos os documentos necessários para a realização da mesma.

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados todos os documentos necessários para que seja avaliada a presente pesquisa. Ou seja: O projeto, carta de encaminhamento com o respectivo termo de aquiescência do departamento e do CIP, orçamento, cronograma, folha de rosto, e o TCLE e o documento dos

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dentes bovinos.

Recomendações:

Seja corrigido o cronograma.

Conclusões ou Pendências e Lista de Inadequações:

A presente pesquisa foi analisada por este CEP na data de 08 de Agosto de 2018 e naquela data a pesquisa foi considerada com pendencias para sua aprovação. A pesquisadora retornou a pesquisa para uma nova análise por este CEP no dia 21 de setembro de 2018. Mais uma vez a pesquisa ficou com pendências uma vez que faltou anexar o termo de aquiescência do CIP-Centro Integrado de Pesquisa, já que seria lá o local em que a pesquisadora iria recrutar um participante para a sua pesquisa. O termo de aquiescência foi anexado e apresentado a este comitê; o que ocorre agora é que a pesquisadora não corrigiu o cronograma. Pelo cronograma apresentado para a análise a fase experimental da pesquisa já teria sido iniciada em setembro/2018 e já estaria terminando no dia 30 de novembro de 2018. A pesquisadora precisa corrigir o cronograma, por isso aprovamos o projeto mas com essa recomendação.

Considerações Finais a critério do CEP:

Esse projeto foi considerado APROVADO na reunião ordinária do CEP de 28/11/2018, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a apresentação de relatório final. Os relatórios parciais deverão estar de acordo com o cronograma e/ou parecer emitido pelo CEP. Alterações na metodologia, título, inclusão ou exclusão de autores, cronograma e quaisquer outras mudanças que sejam significativas deverão ser previamente comunicadas a este CEP sob risco de não aprovação do relatório final. Quando da apresentação deste, deverão ser incluídos todos os TCLEs e/ou termos de doação assinados e rubricados, se pertinentes.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1167640.pdf	03/10/2018 16:07:12		Aceito
Parecer Anterior	ultimoparecer.pdf	03/10/2018 16:06:09	Lyz Canali	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_2909495.pdf	03/10/2018 16:05:52	Lyz Canali	Aceito
Outros	termoaquiescenciacip.pdf	03/10/2018 17:31:52	Lyz Canali	Aceito

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Continuação do Parecer: 3.088.184

Outros	oficiocartaespostapendencias.doc	02/09/2018 19:11:18	Lyz Canali	Aceito
Parecer Anterior	Parecer.pdf	02/09/2018 19:08:57	Lyz Canali	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TermoConsentimentoLivreEsclarecido.pdf	02/09/2018 19:06:52	Lyz Canali	Aceito
Outros	protocolocomiteanimais.docx	02/09/2018 16:10:43	Lyz Canali	Aceito
Outros	termodedoacaodentesbovino.pdf	16/08/2018 16:06:09	Lyz Canali	Aceito
Folha de Rosto	Folhaderosto.pdf	17/07/2018 14:59:00	Lyz Canali	Aceito
Outros	cartadeencaminhamentotermdeaquiescencia.pdf	10/07/2018 23:14:48	Lyz Canali	Aceito
Outros	QuestionarioTecnicoPesquisador.doc	02/07/2018 22:57:14	Lyz Canali	Aceito
Cronograma	Cronograma.docx	02/07/2018 22:54:16	Lyz Canali	Aceito
Projeto Detalhado / Brochura Investigador	Projetodetalhadolyz.doc	02/07/2018 22:53:04	Lyz Canali	Aceito
Declaração de Pesquisadores	DeclaracaoCompromissoPesquisadorResultadosPesquisa.doc	02/07/2018 22:51:43	Lyz Canali	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BAURU, 04 de Dezembro de 2018

Assinado por:
Ana Lúcia Pompéia Fraga de Almeida
(Coordenador(a))

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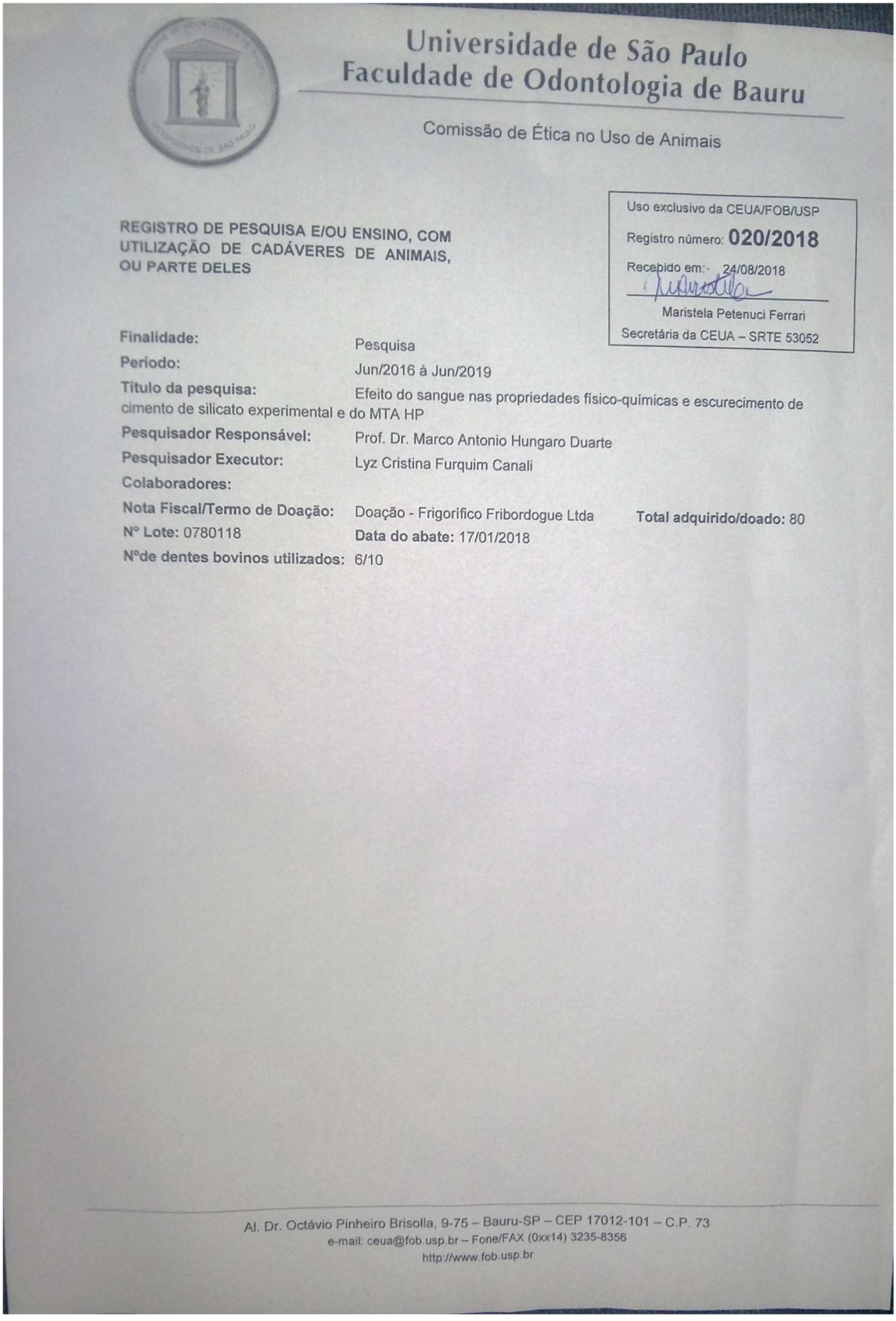
ANNEX B – Registration of research with animals



Universidade de São Paulo
Faculdade de Odontologia de Bauru

Comissão de Ética no Uso de Animais

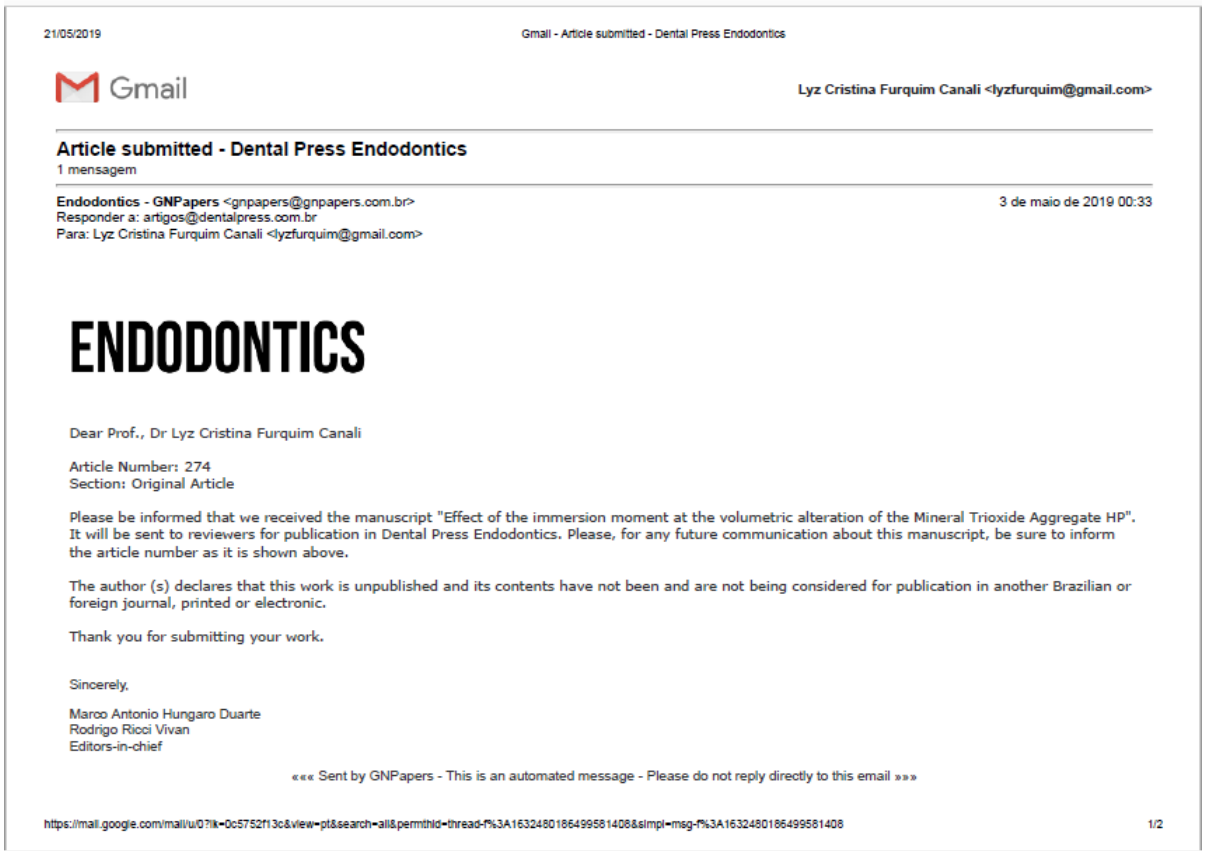
Uso exclusivo da CEUA/FOB/USP
Registro número: **020/2018**
Recebido em: 24/08/2018
Maristela Petenuci Ferrari
Secretária da CEUA – SRTE 53052

REGISTRO DE PESQUISA E/OU ENSINO, COM UTILIZAÇÃO DE CADÁVERES DE ANIMAIS, OU PARTE DELES

Finalidade: Pesquisa
Período: Jun/2016 à Jun/2019
Título da pesquisa: Efeito do sangue nas propriedades físico-químicas e escurecimento de cimento de silicato experimental e do MTA HP
Pesquisador Responsável: Prof. Dr. Marco Antonio Hungaro Duarte
Pesquisador Executor: Lyz Cristina Furquim Canali
Colaboradores:
Nota Fiscal/Termo de Doação: Doação - Frigorífico Fribordogue Ltda **Total adquirido/doado:** 80
Nº Lote: 0780118 **Data do abate:** 17/01/2018
Nºde dentes bovinos utilizados: 6/10

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-101 – C.P. 73
e-mail: ceua@fob.usp.br – Fone/FAX (0xx14) 3235-8356
http://www.fob.usp.br

ANNEX C – Submission confirmation for Dental Press Endodontics



21/05/2019 Gmail - Article submitted - Dental Press Endodontics

Gmail

Lyz Cristina Furquim Canali <lyzfurquim@gmail.com>

Article submitted - Dental Press Endodontics
1 mensagem

Endodontics - GNPapers <gnpapers@gnpapers.com.br> 3 de maio de 2019 00:33
Responder a: artigos@dentalpress.com.br
Para: Lyz Cristina Furquim Canali <lyzfurquim@gmail.com>

ENDODONTICS

Dear Prof., Dr Lyz Cristina Furquim Canali

Article Number: 274
Section: Original Article

Please be informed that we received the manuscript "Effect of the immersion moment at the volumetric alteration of the Mineral Trioxide Aggregate HP". It will be sent to reviewers for publication in Dental Press Endodontics. Please, for any future communication about this manuscript, be sure to inform the article number as it is shown above.

The author (s) declares that this work is unpublished and its contents have not been and are not being considered for publication in another Brazilian or foreign journal, printed or electronic.

Thank you for submitting your work.

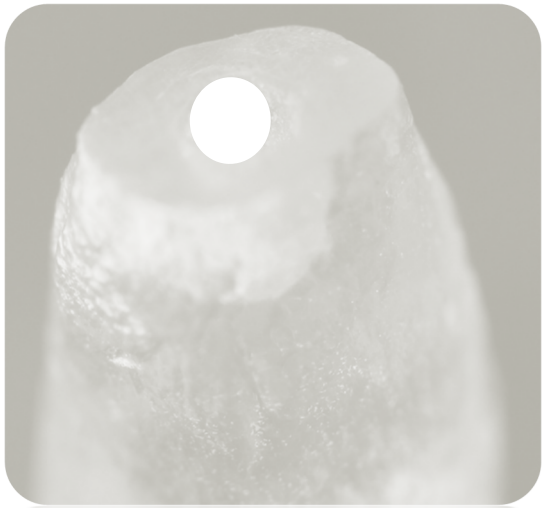
Sincerely,

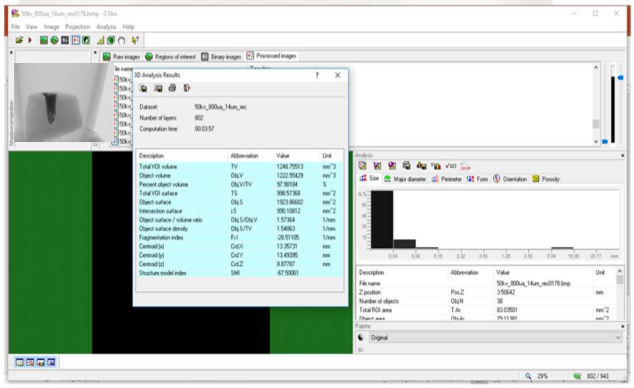
Marco Antonio Hungaro Duarte
Rodrigo Ricci Vivan
Editors-in-chief

«« Sent by GNPapers - This is an automated message - Please do not reply directly to this email »»

https://mail.google.com/mail/u/0?ik=0c5752f13c&view=pt&search=all&permthid=thread-f%3A1632480186499581408&simpl=msg-f%3A1632480186499581408 1/2

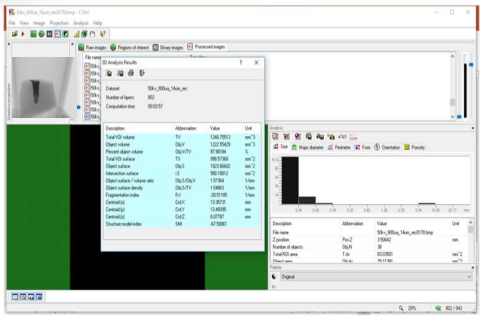
ANNEX D – Illustrative figures of the methodologies of article 1





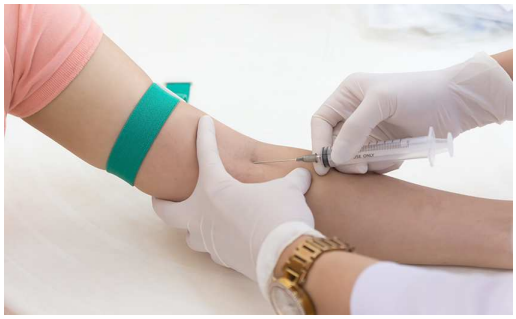
ANNEX E – Illustrative figures of the methodologies of article 2

1- Volumetric alteration



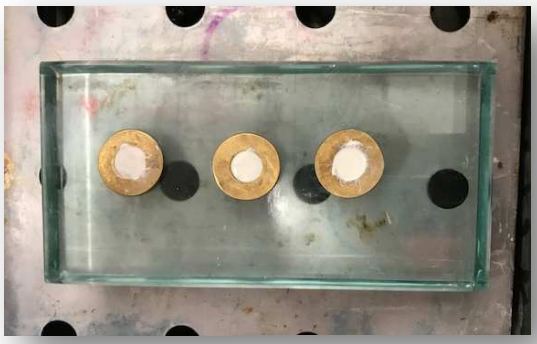
2- pH and calcium ions release

3- Cytotoxicity with release of nitric oxide (NO) when in contact with macrophages

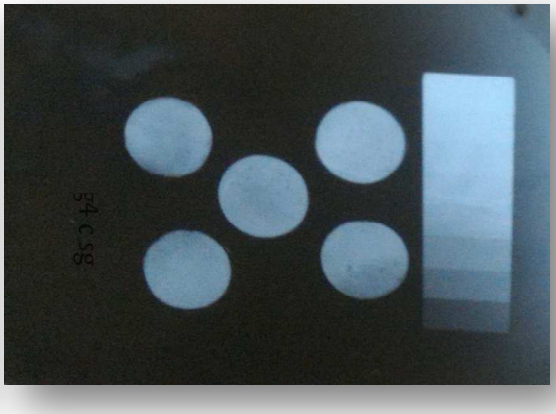


ANNEX F – Illustrative figures of the methodologies of article 3

1- Setting time

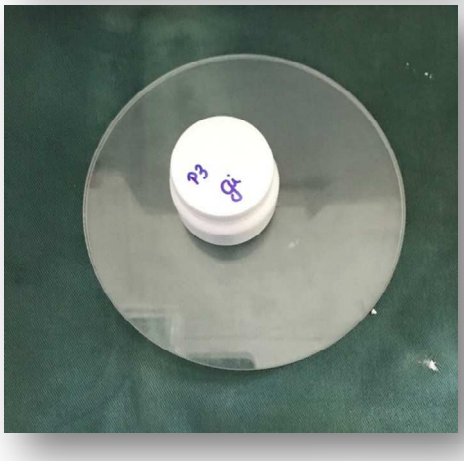


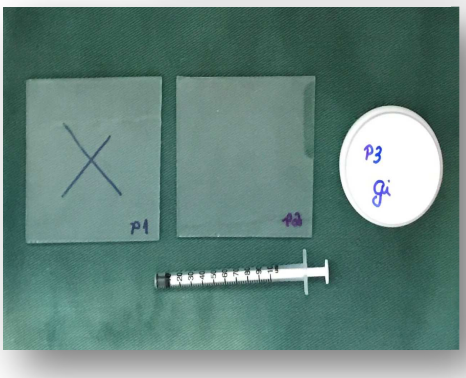
2- Radiopacity

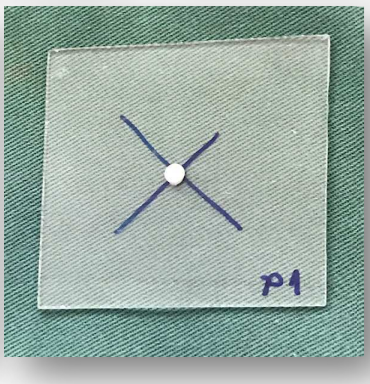


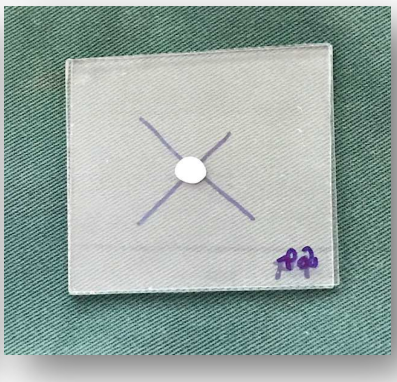
3- Flow and thickness



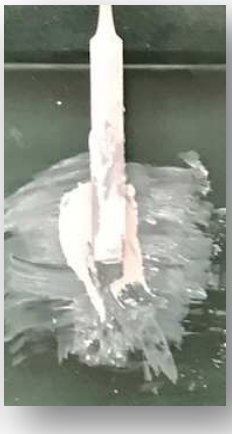








4- Color change

Vita Easyshade (Vita-Zanhnfabrik, Alemanha)

5- Compressive strength