UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

BRUNO MARTINI GUIMARÃES

Evaluation of Sealing ability, color alteration and surface morphology of MTA and Portland cement associated with different radiopacifiers and liquids

Avaliação da capacidade seladora, alteração de cor e de componentes superficiais do MTA e cimento Portland associado à diferentes radiopacificadores e diferentes líquidos

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Orientador: Prof. Dr. Marco Antonio Hungaro Duarte

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FOLHA DE APROVAÇÃO

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ABSTRACT

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Evaluation of Sealing ability, colour alteration and surface morphology of MTA and Portland cement associated with different radiopacifiers and liquids.

This study aimed to analyze the influence of two different vehicles for MTA manipulation on color alteration, chemical characteristics, and radiopacity after immersion in blood or distilled water (DW). In addition, some physical-chemical properties, volumetric solubility and bioactivity of new brands of calcium silicate cements such as MTA Repair HP, MTA Vitalcem and MTA Flow were compared with MTA Angelus. MTA Angelus mixed with 100% Distilled Water (DW) or 80% DW/20% propylene glycol (PG) as vehicles were placed into rubber rings and incubated at 37°C and 100% relative humidity. Color assessment and scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDX) analysis were performed after setting and repeated after 7, 15, and 30 days after immersion in blood and DW. The data were analyzed by using Kruskal-Wallis followed by the Dunn test. The association of 80% DW/20% PG used as vehicle presented significantly lower color alterations compared with 100% DW, when immersed in blood ($p < 0.05$). All samples surfaces displayed loss of bismuth after immersion in both media and consequently a decrease in radiopacity was observed with significant results after 30 days. This study showed that ratio of 80% DW/20% PG as a vehicle for MTA Angelus results in a lower color alteration when in contact with blood. The physical-chemical properties such as radiopacity, calcium release, the pH change, solubility, water sorption, porosity, surface morphology, and bioactivity of MTA Repair HP and MTA Vitalcem were compared with MTA Angelus. Water absorption, interconnected pores and apparent porosity were measured after 24h-immersion in DW at 37ºC. Calcium and pH were tested up to 28d in DW. Morphological and chemical analyses of the material surfaces were performed by using ESEM-EDX after 28d in HBSS. All the materials showed good bioactivity after 28d in HBSS. The data were analyzed by using two-way ANOVA followed by the RM Student-Newman-Keuls test ($p<0.05$). MTA Repair HP and MTA Vitalcem had some positives properties, such as extended alkalinizing activity and calcium release that favored the bioactivity. However, MTA Vitalcem showed scant radiopacity, long setting time and high solubility. The radiopacifier Calcium Tungstate, present in MTA Repair HP can be used to replace the traditional Bismuth Oxide. In

relation to the comparison between MTA Angelus and MTA Flow, it was analyzed the pH, volumetric change, radiopacity and bioactivity. In the volumetric change test, the root-end fillings of 20 acrylic teeth with both materials were scanned twice by a Micro-CT, before and after immersion in DW for 168 h. The morphological and bioactivity of the materials were performed by using SEM-EDX after 28d in HBSS. The data were analyzed by using two-way ANOVA followed by the RM Student-Newman-Keuls test ($p<0.05$). MTA Angelus showed statistically higher radiopacity values ($p<0.05$). the results pointed that the new cement MTA Flow showed good physical-chemical characteristics and bioactivity, showing values similar to those of MTA Angelus.

Keywords: Calcium Silicate; Materials Testing; Endodontics; Scanning Electron Microscopy.

RESUMO

RESUMO

Avaliação da capacidade seladora, alteração de cor e de componentes superficiais do MTA e cimento Portland associado à diferentes radiopacificadores e diferentes líquidos.

O objetivo do estudo foi analisar a influência de dois diferentes veículos utilizados na manipulação do MTA em relação a alteração de cor, características superficiais e radiopacidade, antes de depois do contato com sangue ou Água Destilada (AD). Adicionalmente, foram analisadas as propriedades físico-quimicas, solubilidade volumétrica e a bioatividade de novas marcas comerciais de cimentos de silicato de cálcio como MTA Repair HP, MTA Vitalcem e MTA Flow comparados com o MTA Angelus. Foram realizadas associações do MTA Angelus com 100% de Água Destilada (AD) e outra com 80% de AD e 20% de Propilenoglicol (PG) como veículos e, após isso, estas foram inseridas em anéis de borracha e encubadas a 37ºC e 100% de humidade. A análise de cor e realização do escaneamento da amostra em microscópio eletrônico de varredura (MEV) juntamente com a espectroscopia de energia dispersiva (EDX) foram realizadas após a presa e depois de 7, 15 e 30 dias após a imersão em sangue ovino e água destilada. Os dados foram submetidos aos testes não paramétricos de Kruskal-Wallis e Dunn. A associação com o veículo de 80% AD com 20% de PG apresentou alteração de cor significativamente menor quando comparada com o veículo de 100% de AD quando imersos em sangue ($p < 0.05$). Em todas as amostras a análise superficial comprovou a perda de partículas de bismuto durante a imersão nos diferentes líquidos e consequentemente a redução da radiopacidade apresentando resultados significativos após 30 dias. Esse estudo demonstrou que o uso do veículo 80% de AD com 20% de PG no MTA Angelus apresentou menor alterações de cor quando em contato com sangue. As propriedades físico químicas como radiopacidade, liberação de cálcio, pH, solubilidade, absorção de água, porosidade, análise das características superficiais e bioatividade dos cimentos MTA Repair HP e MTA Vitalcem foram comparadas com as do MTA Angelus. A absorção de água e a porosidade aparente foram analisadas 24h após a imersão dos espécimes em AD a 37ºC. A liberação e cácio e o pH foram anaisados após 28d de imersão em AD. As características superficiais e a Bioatividade foram analisadas

em MEV/EDX após 28 dias de imersão em HBSS. Os dados foram submetidos aos testes de two-way ANOVA seguido por RM Student-Newman-Keuls ($p<0.05$). Todos os materiais apresentaram boa bioatividade após 28 dias de imersão em HBSS. O MTA Repair HP e o MTA Vitalcem apresentaram boa atividade alcalinizante e liberação de ions cálcio favorecendo a bioatividade. Entretanto o MTA Vitalcem mostrou baixa radiopacidade, longo tempo de presa e alta solubilidade. O radiopacificador Tungstato de Cálcio, utilizado no MTA Repair HP pode ser usado como substituto do tradicional óxido de bismuto. Em relação a comparação entre o MTA Angelus e o MTA Flow, foram realizados as analises de pH, alteração volumétrica, radiopacidade e bioatividade. Em relação a alteração volumétrica, 20 retro-cavidades de dentes de acrílico preenchidas com os materiais foram escaneadas duas vezes em um aparelho de Micro-CT antes e após a imersão por 168h em AD. As características superficiais e a bioatividade foram realizadas por MEV/EDX depois de 28d de imersão em HBSS. Os dados foram submetidos aos testes de two-way ANOVA seguido por RM Student-Newman-Keuls ($p<0.05$). O MTA Angelus apresentou estatisticamente maior radiopacidade ($p<0.05$). Os resultados apontaram que o novo cimento MTA Flow apresentou boas características físico-quimicas e bioatividade similares ao MTA Angelus.

Palavras chave: Silicato de Calcio; Teste de Materiais; Endodontia; Microscopia Eletrônica de Varredura.

LISTA DE ABREVIATURA E SIGLAS

%	percentagem
+	mais
=	igual
MTA	Mineral Trioxide Aggregate
PG	Propylene glycol
DW	Distillated Water
AD	Água Destilada
SEM	Scanning Electron Microscope
pH	Potential of Hydrogen
EDX	Energy-dispersive X-ray spectroscopy
BMP-2	Bone morphogenetic protein 2
MEV	Microscopia Eletrônica de Varredura
SBF	Simulated body fluid
mm^3	milímetro cubico
n	numero
C	carbon
N	nitrogen
O	oxygen

SUMMARY

1 INTRODUCTION

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Calcium silicate-based materials, commonly known in dentistry by the term Mineral Trioxide Aggregate (MTA) is a dental material containing Portland cement blended with a radiopaque powder (MOISEIWITSCH, 1993). Due to its excellent biological and satisfactory physicochemical properties, MTA is considered the ideal material for use as a root-end filling and for repair of root perforations (GANDOLFI et al., 2010). However, it is currently used for a variety of applications including pulp capping, apexification procedures, as a dressing over pulpotomies, and many other endodontic procedures (GUIMARÃES et al., 2015; MENTE et al., 2010).

MTA was conceived by mixing a grey Portland cement with bismuth oxide and used for endodontic applications (MOISEIWITSCH, 1993; TORABINEJAD & WHITE, 1995). The original MTA contained the same tri- and dicalcium silicate major phases as Portland cement, with about 20 % bismuth oxide. The first commercial MTA was introduced as tooth-filling material in 1995 by Torabinejad and White as grey ProRoot® MTA (Dentsply, Tulsa Dental, Johnson City, TN, USA). In 2002, Carolyn Primus introduced and later patented the “tooth-colored ProRoot MTA” (PRIMUS, 2004), a material often designated in the literature as white MTA. The grey and white versions of ProRoot MTA have similar compositions, but the tooth-coloured ProRoot MTA has less iron, roughly 5 versus 0.5 % iron oxide, respec- tively, as observed using energy-dispersive spectroscopy (EDX) (CAMILLERI et al., 2005).

MTA is considered a bioactive cement. The bioactivity, which is, the capacity to produce spontaneously an apatite layer when in contact with phosphate-containing physiological fluids is largely attributable in relation of calcium ions releasing and maintaining a high pH during a long period of time (PRATI & GANDOLFI, 2015). Calcium ion release, alkaline pH and other properties like high radiopacity, high sealing ability, adaptation to the dentinal walls and antimicrobial activity are important characteristics of MTA and Portland cement (PARIROKH & TORABINEJAD, 2010a; TORABINEJAD et al., 1993). The combination of all these characteristics makes it the retrograde obturation and perforation repair material of choice in endodontic surgery.

MTA is a hydraulic material, and thus the hydration process depends on water. The manufacturers of MTA recommend mixing with distilled water, which results in a sandy and dry material (KOGAN et al., 2006). With the aim to improve the manipulation, some substances including water-soluble polymer (CAMILLERI & MALLIA, 2011), and a gel (GOMES-FILHO et al., 2009) have been tested in order to establish their efficacy in enhancing the cement manipulation.

Propylene glycol (PG) is frequently used in Dentistry as a vehicle for calcium hydroxide and has also been tested as an additive to improve MTA mixing (BRITO-JÚNIOR et al., 2010; DUARTE et al., 2012; SALEM MILANI et al., 2013). Duarte et al. showed that the ratio of 80% Distillated Water (DW) – 20% PG increased the setting time, improved flowability and improved the pH and calcium ion release at the initial periods of the experiment (DUARTE et al., 2012). The effect of PG as a vehicle of MTA on the color alteration is unknown.

Dental discoloration has been reported following application of MTA (BELOBROV & PARASHOS, 2011; FELMAN & PARASHOS, 2013). Bismuth oxide has been hypothesized as the component responsible for the color alteration of MTA and consequently tooth discoloration (MARCIANO et al., 2014; VALLÉS et al., 2013a, 2013b). Camilleri reported that the interaction of MTA with sodium hypochlorite, which is used regularly for irrigation in Endodontics resulted in a black MTA surface. The bismuth oxide reacted with sodium hypochlorite forming bismuth carbonate, which is light sensitive (CAMILLERI, 2014). The use of alternative radiopacifiers to bismuth oxide in MTA has also been tested (CUTAJAR et al., 2011). Reports that bismuth oxide interferes with MTA hydration (CAMILLERI, 2007) and causes deterioration in the mechanical properties (COOMARASWAMY et al., 2007), have encouraged research on the substitution of bismuth oxide by other radiopacifiers.

Zirconium oxide has been tested as a substitute to bismuth oxide due to its adequate radiopacity (CAMILLERI et al., 2013; HÚNGARO DUARTE et al., 2009) and no interference with hydration mechanism of Portland cement (CAMILLERI et al., 2011). Moreover, this radiopacifier does not interfere in the deposition of hydroxyapatite (CAMILLERI et al., 2013) and release of calcium ions (CAMILLERI et al., 2011). Calcium tungstate is another radiopacifier that has been investigated, and shows adequate physical and chemical properties when associated with Portland cement (HUNGARO DUARTE et al., 2012; HÚNGARO DUARTE et al., 2009). These alternative radiopacifiers do not show discoloration in contact with collagen (MARCIANO et al., 2014) and were not completely analyzed the influence related to bioactivity.

Moreover, dental discoloration resulting from the interaction of the cement with tissue fluids like blood is detrimental especially in esthetic regions (MARCIANO et al., 2014). In this context, the interaction of blood with MTA when used for vital pulp therapy revascularization or perforation repair has also been implicated with discoloration (FELMAN & PARASHOS, 2013). The mechanisms of how PG as a vehicle of MTA and blood contamination influences the discoloration process are currently unknown (FELMAN & PARASHOS, 2013).

Recently, new formulations have been introduced to the market (GANDOLFI et al., 2015, 2014, 2012; SINGH et al., 2015; SOUZA et al., 2015). Among them, MTA Repair HP (Angelus, Londrina, PR, Brazil), MTA Vitalcem (School of Dentistry, Catholic University of Santa María, Peru) and MTA Flow (Ultradent Products Inc, South Jordan, UT, USA) have been proposed. MTA Repair HP is based on the formulation of MTA Angelus but contains Calcium Tungstate as radiopacifier and a new mixing liquid with a plasticizer agent. Silva et al. showed that the new formulation presented better push-out bond strength than its predecessor, White MTA Angelus (SILVA et al., 2016).

MTA Vitalcem cement has a composition similar to that of conventional MTA but contains Zirconium Dioxide as radiopacifier. It has shown antimicrobial properties (OBANDO PEREDA et al., 2009) and regenerating properties (FIGUEROA & OBANDO, 2014) similar to those of MTA Angelus. MTA Flow™ (Ultradent Products Inc, South Jordan, UT, USA) is a novel MTA-like cement composed of a grey powder of di- and tricalcium silicate that sets with a water-based gel that makes the cement more washout-resistant and also gives a variety of consistency. Bismuth Oxide is the radiopacifier of MTA Flow. In the literature at present, there are no studies on the physical-chemical characterization of those new cements.

Therefore, the aim of this work was to study and analyze if some modifications made on MTA's formulation (such as vehicles and radiopacifiers) could improve its chemical-physical properties and bioactivity trying to find new enhanced materials that are better for clinicians and consequently its patients.

For this purpose, the study was performed in three steps:

1. The discoloration of MTA can be exacerbated by the interaction of the cement with body fluids such as blood. The study aimed to analyze the influence of two different vehicles for MTA manipulation on color alteration, chemical characteristics, and radiopacity after immersion in blood or distilled water (DW);
2. Analyze the chemical-physical properties such as radiopacity, calcium release, the pH change, solubility, water sorption, porosity, surface morphology, and apatite-forming ability (bioactivity) of 2 new calcium silicate-based materials containing new radiopacifiers (Calcium Tungstate and Zirconium Oxide);
3. Analyze the chemical-physical properties: pH, volumetric change, radiopacity and apatite-forming ability (bioactivity) of a new tricalcium silicate material MTA Flow.

2 ARTICLES

2 ARTICLES

2.1 Article 1 - Color Stability, Radiopacity, and Chemical Characteristics of White Mineral Trioxide Aggregate Associated with 2 Different Vehicles in Contact with Blood

Color Stability, Radiopacity, and Chemical Characteristics of White Mineral Trioxide Aggregate Associated with 2 Different Vehicles in Contact with Blood

Bruno Martini Guimarães, DDS, MSc, Talita Tartari, DDS, MSc,* Marina Angélica Marciano, DDS, PhD,* Rodrigo Ricci Vivan, DDS, PhD,* Rafael Francisco Lia Mondeli, DDS, PhD,* Josette Camilleri, DDS, PhD,† and Marco Antonio Hungaro Duarte, DDS, PhD**

Abstract

Introduction: Discoloration of mineral trioxide aggregate (MTA) can be exacerbated by the interaction of the cement with body fluids such as blood. This study aimed to analyze the color alteration, chemical characteristics, and radiopacity of MTA manipulated with 2 different vehicles after immersion in blood or distilled water (DW). **Methods:** MTA mixed with 100% DW or 80% DW/20% propylene glycol (PG) as vehicles were placed into rubber rings and incubated at 37°C and 100% relative humidity until set. Color assessment and scanning electron microscopy/energy-dispersive spectroscopy analysis were performed after setting and repeated after 7, 15, and 30 days after immersion in blood and DW. Statistical analysis for color alteration and radiopacity was performed using nonparametric Kruskal-Wallis and Dunn tests ($P < .05$). **Results:** When 80% DW/20% PG was used as the vehicle, significantly lower color alterations were observed for all time periods compared with 100% DW when immersed in blood ($P < .05$). All surfaces displayed morphologic changes after immersion in both media because of loss of bismuth. A decrease in radiopacity was observed over time in all groups, with a statistically significant difference after 30 days for groups DW immersed in blood and 80% DW/20% immersed in both media ($P < .05$). **Conclusions:** The ratio of 80% DW/20% PG as a vehicle for MTA results in a lower color alteration when in contact with blood. *(J Endod 2015;41:947–952)*

Key Words

Blood, characterization, color stability, tooth discoloration, white mineral trioxide aggregate

Mineral trioxide aggregate (MTA) is a calcium silicate–based cement containing bismuth oxide as a radiopacifier (1). It was developed as a root-end filling material but is also used for vital pulp therapies, treatment of root fractures, perforations, and apexifications (2–4). Dental discoloration has been reported after the application of MTA (5, 6). The interaction of MTA with sodium hypochlorite, which is used regularly for irrigation in endodontics resulted in a black MTA surface. The bismuth oxide reacted with sodium hypochlorite forming bismuth carbonate, which is light sensitive (7). The influence of light on the color stability of MTA has been reported (8). Dental staining resulting from the interaction of the cement with dental hard tissues and tissue fluids is detrimental, especially in esthetic regions (3).

The contamination of MTA by blood has been investigated in a number of laboratory studies in terms of the effect on its physical properties (9), leakage (10), displacement (11), and marginal adaptation (12). Blood contamination has a detrimental effect on the chemical properties of MTA, resulting in a lack of formation of the crystalline calcium hydroxide in the early stage of the hydration process (9). The interaction of blood with MTA when used for vital pulp therapy revascularization or perforation repair has also been implicated with discoloration (6). The mechanisms of how blood contamination exacerbates the discoloration process are currently unknown (6).

Propylene glycol (PG) is frequently used in dentistry as a vehicle for calcium hydroxide and has also been tested as an additive to improve MTA mixing (13–16). The ratio of 80% distilled water (DW)/20% PG increased the setting time, improved flowability, pH, and calcium ion release in the initial periods of the experiment (15). The effect of PG on the color alteration of MTA is unknown.

The aim of this investigation was to analyze color alteration, chemical characteristics, and radiopacity of MTA Angelus (Londrina, PR, Brazil) manipulated with different vehicles after immersion in blood or DW for different periods of time.

Materials and Methods

White MTA (Angelus) was mixed with 100% DW or 80% DW/20% PG as vehicles (15). The powder/liquid ratio was the same for all groups (1 g powder to 0.3 mL liquid).

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After setting, the materials were distributed according to the immersion solution, which was manual defibrinated sheep blood (to avoid coagulation) as described by Yeh et al (17) and DW, both allocated inside culture plates in a quantity of 3 mL (Corning Inc, New York, NY) according to the following groups:

1. MTA + 100% DW immersed in blood
2. MTA + 100% DW immersed in DW
3. MTA + 80% DW/20% PG immersed in blood
4. MTA + 80% DW/20% PG immersed in DW

Color Assessment

The cements were mixed and placed into rubber rings, with a 10-mm internal diameter and 1-mm height. The rings filled with the materials were stored in an incubator to completely set at 37°C and 100% relative humidity for 2 hours (15).

Cement samples (n = 10) were demolded, and the baseline color of the specimens was determined using a spectrophotometer (Vita Easyshade; Vita Zahnfabrik H. Rauter, Bad Säckingen, Germany) against a white Teflon (DuPont, Habia, Knivsta, Sweden) background to obtain the values of L* (lightness, from 0 = black up to 100 = white), a* (from a = green up to +a = red), and b* (from b = blue up to + b = yellow). Three measurements were performed for each sample. The measurements for each group were repeated after 7, 15, and 30 days of immersion in blood and DW allocated inside culture plates.

CIELab color changes (ΔE) were determined according to the Commission Internacionale I'Eclairage (International Commission on Illumination) by calculating the distance between 2 points by using the following formula:

$$\Delta E* = \left[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right] 1/2$$

Delta values were calculated based on the difference between the final and baseline values for each coordinate (L*, a*, and b*), and values ≥3.3 were considered clinically unacceptable (18).

Scanning Electron Microscopy/Energy-dispersive X-ray Spectroscopy

All disk samples were also examined by scanning electron microscopy (SEM) (Aspex Express; Fei Europe, Eindhoven, Netherlands) at an accelerating voltage of 15–20 kV after setting and after 7, 15, and 30 days of immersion in blood or DW. At each time point, cement disks were placed on aluminum stubs using adhesive carbon discs and analyzed uncoated. Scanning electron micrographs of the sample surfaces at different magnifications were captured. Elemental analysis by energy-dispersive X-ray spectroscopy (EDX), which is fully integrated to the Aspex Express SEM (Fei Europe, Eindhoven, The Netherlands), was conducted over the entire area to determine the elemental composition of the samples. The specimens were then returned to their respective soaking solutions until further testing. A fresh blood drop was also examined by SEM/EDX.

Radiopacity

Six samples were selected for each group having a diameter and thickness according to ISO 6876:2012 and confirmed using a digital caliper (Mitutoyo Corp, Tokyo, Japan). Radiographs were acquired after setting and after 7, 15, and 30 days of immersion in blood or DW by directly placing the specimens of each group on a 57 × 76-mm Ultra-speed occlusal radiographic film (Eastman Kodak

TABLE 1. Score Values for Color Alteration and Luminosity in the Different Groups Evaluated after Immersion in Blood and Distilled Water

		ΔE			Luminosity			
Groups	Immersion substances	T07, median (min–max)	T15, median (min–max)	T30, median (min–max)	T0, median (min–max)	T07, median (min–max)	T15, median (min–max)	T30, median (min–max)
MTA/DW	Blood	72612.88 (31600.39–208049.62)^{Aa}	68419.58 (38124.22–206688.62)^{Aa}	374.85 (265.00–626.50)^{Ab}	17.00 (16.40–19.90)^{Aa}	4.20 (2.80–7.60)^{Bb}	3.9 (3.20–7.30)^{Bb}	4.55 (3.30–7.20)^{Bb}
	DW	0.35 (0.25–0.63)^{Bb}	1.27 (0.50–2.85)^{Ba}	1.95 (0.50–6.73)^{Aa}	17.00 (16.40–17.30)^{Abc}	16.95 (16.40–17.20)^{Ac}	18.40 (17.90–18.70)^{Aa}	17.90 (16.30–19.80)^{Aab}
MTA/80% DW and 20% PG	Blood	15.62 (9.32–19.08)^{Ba}	15.80 (13.23–19.03)^{Ba}	1.65 (0.30–2.20)^{Bb}	15.70 (15.10–15.90)^{Ba}	10.30 (9.90–11.90)^{Ab}	10.40 (10.00–11.30)^{Ab}	10.65 (9.90–11.10)^{Ab}
	DW	0.88 (0.40–1.82)^{Ab}	2.59 (2.01–8.12)^{Aa}	4.02 (2.86–5.94)^{Aa}	15.75 (15.10–15.90)^{Bc}	16.30 (16.00–16.90)^{Bbc}	17.90 (17.80–19.10)^{Aab}	18.15 (17.40–20.60)^{Aa}

ΔE, color alteration; DW, distilled water; L, luminosity; Max, maximum; Min, minimum; MTA, mineral trioxide aggregate; T0, final setting time; T07, 7 days; T15, 15 days; T30, 30 days.

Kruskal-Wallis test, $P < .05$, different capital letters in columns indicate statistically significant differences between MTA prepared using different vehicles in the same time interval and in the same immersion substance.

Friedman test, $P < .05$; different lowercase letters in rows indicate statistically significant intragroup differences between the periods analyzed.

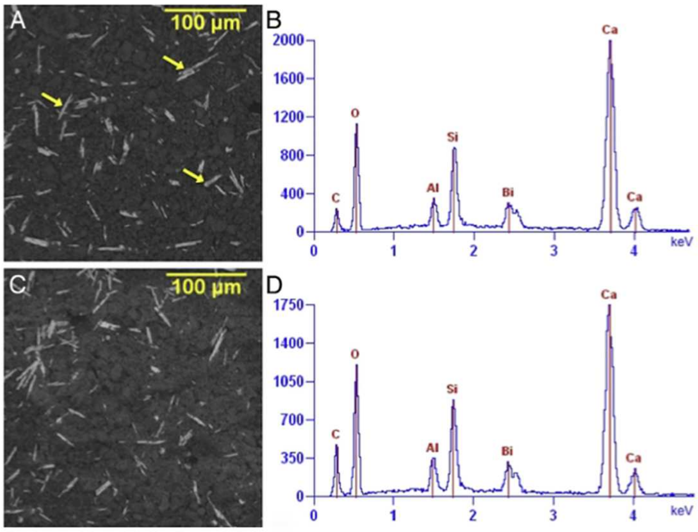

Figure 1. SEM/EDX analyses of MTA prepared with (*A* and *B*) 100% DW and (*C* and *D*) 80% DW/20% PG as the vehicle immediately after cement setting. (*A*) Elongated particles constituting mainly bismuth (*yellow arrows*) were interspersed together with the cement particles. (*B* and *D*) EDX analysis of the area displayed C, O, Al, Si, Bi, and Ca peaks for both vehicles.

Co, Rochester, NY) adjacent to an aluminum step wedge (6063 alloy, 98% purity) ranging from 2.0–16.0 mm in thickness (in 2-mm increments). All specimens were placed at a 30-cm focus film distance for 0.3 seconds at 10 mA and a tube voltage of 65 ± 5 kV in a radiographic unit (Gnatus XR 6010; Gnatus, São Paulo, Brazil). The radiographs were digitized and analyzed using Digora 1.51 software (Soredex, Helsinki, Finland). Radiopacity was determined according to Hungaro Duarte et al (19).

Statistical Analysis

Analysis of color assessment and radiopacity data were assessed using the Shapiro-Wilk test and showed abnormal distribution. The

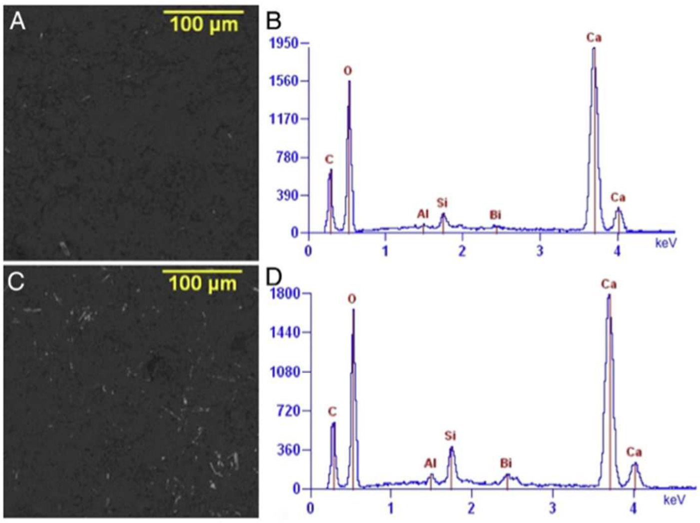

Figure 2. SEM/EDX analyses of MTA prepared with (*A* and *B*) 100% DW and (*C* and *D*) 80% DW/20% PG as the vehicle after 30 days of immersion in DW. (*A* and *C*) External surfaces exhibited a loss of bismuth particles for both vehicles. EDX showed lower peaks of Al, Si, and Bi for both vehicles.

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color alteration, luminosity, and radiopacity (in millimeter Al) values for each group in the different periods of time were compared using Friedman analysis of variance ($\alpha = 0.05$). The Kruskal-Wallis test with Dunn post hoc ($\alpha = 0.05$) was applied to identify differences in color alteration, luminosity, and radiopacity to MTA prepared using different vehicles for the same period of time and in the same immersion substance.

Results

Color Assessment

Table 1 presents the median, maximum, and minimum values of color alteration (ΔE) for all groups after 7, 15, and 30 days and luminosity before and after 7, 15, and 30 days of immersion in blood and DW. The greatest color alterations were observed in the groups immersed in blood. These groups presented significant decreases in luminosity after 7 days ($P < .05$), showing discoloration above the clinically accepted level ($\Delta E \geq 3.3$). However, this color alteration was significantly less at 7 and 15 days than at 30 days of immersion for both groups ($P < .05$).

PG as a vehicle (80% DW/20% PG) presented significantly lower color alteration for all time periods when immersed in blood and showed clinically acceptable levels of color alteration (ΔE values $\leqq$ 3.3) after 30 days.

In the DW groups, luminosity increased over time with significant differences in both groups between the initial luminosity and after 15 days of immersion ($P < .05$). This difference was maintained after 30 days in the 80% DW/20% PG group. The ΔE was significantly higher for this same group at 7 and 15 days ($P < .05$); however, this difference between the groups was not observed after 30 days of immersion ($P > .05$).

SEM-EDX Analysis

Selected photomicrographs and corresponding EDX spectra of elements obtained from the samples before and after immersion in blood or DW for different periods of time are presented in Figures 1–3. Morphologic changes were noted in all surfaces after immersion in both substances. MTA Angelus comprised cement particles rich in calcium and silicon with traces of aluminum. In initial photomicrographs (Fig. 1*A* and *C*), elongated particles constituting mainly bismuth (Fig. 1*A*) interspersed with cement particles could be observed for both vehicles.

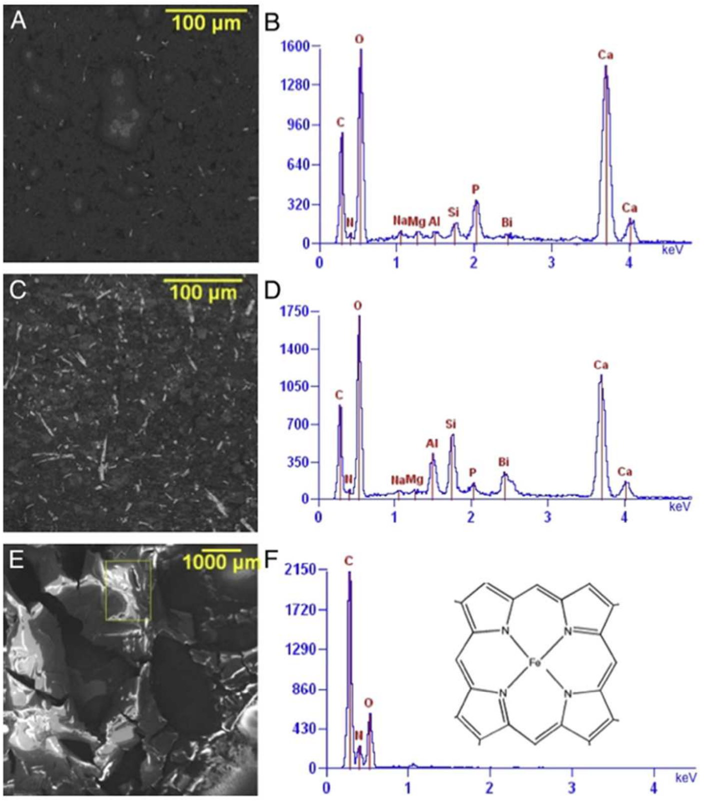

Figure 3. SEM/EDX analyses of MTA prepared with (*A* and *B*) 100% DW and (*C* and *D*) 80% DW/20% PG as the vehicle after 30 days of immersion in blood. (*A* and *C*) External surfaces exhibited a loss of bismuth particles for both vehicles. (*B* and *D*) EDX analysis displayed the appearance of N, Na, Mg, and P peaks for both vehicles. (*E* and *F*) SEM/EDX analysis of a blood drop. (*F*) EDX spectra displayed C, N, and O peaks that may be related to the porphyrin from the heme group molecule.

SEM-EDX analysis of MTA prepared with 100% DW and 80% DW/20% PG as vehicles immediately after cement setting are shown in Figure 1A and B and Figure 1C and D, respectively. MTA Angelus manipulated with both vehicles revealed a rough cement surface before immersion (Fig. 1A and C). EDX revealed C, O, Al, Si, Bi, and Ca peaks, independently of the vehicles used (Fig. 1C and D).

After 30 days of immersion in DW, the external surface appeared to be more regular with a reduction of bismuth particles in both vehicles, corroborated by radiopacity analysis, in which the same cement pellet was used (Fig. 2A and C). EDX showed C, O, and Ca peaks and a reduction of Al, Si, and Bi in both vehicles (Fig. 2B and D).

After 30 days of immersion in blood, external surfaces were more regular and also presented a reduction of bismuth particles in both vehicles used. EDX revealed a reduction of Si and increased O content (Fig. 3B and D). EDX analysis displayed the appearance of N, Na, Mg, and P peaks in an increasing order (Fig. 3B and D). The peaks of Ca, Si, and P suggest the presence of calcium phosphate deposits. SEM/EDX analysis of a fresh blood drop is represented in Figure 3E and F, respectively. EDX showed C, N, and O peaks.

Radiopacity

Table 2 presents the median, maximum, and minimum radiopacity values expressed as aluminum equivalent millimeters (Al mm) for all groups before and after 7, 15, and 30 days of immersion in blood and DW. All groups presented a decrease in radiopacity over time with statistical differences after 30 days for groups MTA/DW immersed in blood and MTA/80% DW/20% PG immersed in blood and DW ($P < .05$).

A statistically significant lower radiopacity was observed after 7 days of immersion in blood compared with immersion in DW ($P < .05$). For samples immersed in DW, a statistically significant lower radiopacity was observed after 30 days in the 80% DW/20% PG group ($P < .05$).

Discussion

Color is 1 of the most important factors to be considered in esthetic dentistry. The use of calcium silicate–based cements in vital pulp therapy, such as pulpotomy, revascularization, and direct pulp capping, can lead to cement alterations and consequent dentin discoloration (5, 13). Sphere spectrophotometers, such as that used in this study, provide a more accurate assessment of color change than subjective human evaluation (3, 20, 21).

As expected, the major color alterations were observed in the groups immersed in blood, presenting decreases in luminosity after 7 days in both vehicles. In this study, PG was used in a proportion suggested by Duarte et al (15) to improve MTA mixing and to analyze the influence on cement color change. In the 80% DW/20% PG group immersed in blood, the color alteration was lower for all time periods and clinically acceptable (18) after 30 days. For the groups immersed in DW, it was observed that luminosity increased over time probably because of dissociation, in immersion solution, of bismuth oxide (22, 23); this is considered to be the component responsible for color alteration of MTA (8, 24).

The characterization of the materials was performed using SEM-EDX, which is a well-documented method for evaluating the composition of endodontic materials (25–27). Scanning electron micrographs of the cements showed changes in microstructural characteristics, which are more evident in samples immersed in blood.

Red blood cells are known tooth staining agents (28). The contact with MTA may exacerbate discoloration by the incorporation of blood elements (6). Lenherr et al (29) suggest that the porosities of the material may uptake blood components and may be responsible for the observed discoloration. EDX analysis displayed the appearance of nitrogen peaks only when the cements were immersed in blood as shown in Figure 3B and D, which could be related to the absorbance of the heme group present in hemoglobin (30).

The heme group (molecule attached with EDX Spectra in Fig. 3F) is a prosthetic group consisting of a ferrous (Fe^{2+}) ion contained in the center of a large heterocyclic organic ring, called porphyrin, made up of 4 pyrrolic groups joined together by methine bridges (30). SEM/EDX analysis of a fresh blood drop is represented in Figure 3E and F. EDX showed C, N, and O peaks that may be related to porphyrin from the heme group of the blood. Over time, blood that is in contact with the cement undergoes a natural redox reaction causing the loss of Fe^{2+} (which possesses a red color) and becomes ferric (Fe^{3+}), which possesses a dark brown color that may result in a darkening of the cement and consequently tooth discoloration (6).

Radiopacity is an important physical property required for cement (31). A low radiopacity value is essential to identify the material in the root canal and allows filling failures to be corrected before final restoration (19). The analysis of the results showed that all groups presented a decrease in radiopacity over time regardless of the immersion substance. The lower values of radiopacity observed may be related to a higher dissociation of bismuth oxide (22, 23).

Previous investigations have already showed that bismuth oxide is related to color alteration of MTA (3, 7, 8). Therefore, a decrease in bismuth concentration and consequently lower radiopacity values corroborate with our color assessment results; this is because luminosity increased and color alteration caused by immersion decreased over time (22).

Conclusion

Although MTA discoloration has been associated with its radiopacifier (ie, bismuth), the oxidation of the heme group present in hemoglobin can exacerbate this discoloration. The ratio of 80% DW/20% PG as a vehicle results in lower color alteration when in contact with blood. This can be recommend in cases in which esthetics are crucial and

TABLE 2. Scores Values for the Radiopacity (in Al mm) in the Different Groups Evaluated after Immersion in Blood and Distilled Water

Groups	Immersion	Radiopacity T0, median (min–max)	T07, median (min–max)	T15, median (min–max)	T30, median (min–max)
MTA/100% DW	Blood	7.75 (7.09–8.80)^{Aa}	5.37 (5.22–5.71)^{Bb}	6.85 (5.62–7.46)^{Aab}	6.26 (5.43–6.74)^{Ab}
	DW	8.94 (7.69–10.30)^{Aa}	6.17 (5.94–6.21)^{Ab}	6.81 (6.63–7.05)^{Aa}	6.59 (6.37–6.63)^{Aab}
MTA/80% DW + 20% PG	Blood	9.45 (6.60–9.83)^{Aa}	6.28 (5.97–7.68)^{Aab}	6.58 (5.65–8,20)^{Aab}	5.65 (5.29–6.45)^{Ab}
	DW	7.46 (7.05–8.87)^{Ba}	6.15 (5.74–6.85)^{Ab}	6.81 (5.41–6.97)^{Aab}	5.14 (5.25–6.29)^{Bb}

Al mm, Aluminum equivalent millimeters; DW, distilled water; Max, maximum; Min, minimum; MTA, mineral trioxide aggregate; PG, propylene glycol; T0, final setting time; T07, 7 days; T15, 15 days; T30, 30 days.

Kruskal-Wallis test, $P < .05$; different capital letters in columns indicate statistically significant differences between MTA prepared using different vehicles in the same time interval and in the same immersion substance.

Friedman test; $P < .05$; different lowercase letters in rows indicate statistically significant intragroup differences between the periods analyzed.

blood contamination is likely, such as coronal perforations, pulp capping, and coronal sealing in revascularization, to achieve clinically acceptable levels of color alteration.

Acknowledgments

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The authors deny any conflicts of interest related to this study.

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2.2 Article 2 - Physico-chemical properties of calcium silicate-based formulations MTA Repair HP and MTA Vitalcem

The submitted article presented in this Thesis was written according to the Journal of Applied Oral Science *instructions and guidelines for article submission*

ABSTRACT

Objective: The study aimed to analyze the following chemical-physical properties: radiopacity, calcium release, the pH change, solubility, water sorption, porosity, surface morphology, and apatite-forming ability of 2 new calcium silicate-based materials. Material and Methods: MTA Repair HP and MTA Vitalcem were tested in comparison with MTA Angelus. Radiopacity and final setting time were analyzed. Water absorption, interconnected pores and apparent porosity were measured after 24h-immersion in deionized water at 37ºC. Calcium and pH were tested up to 28d in deionized water. The data were analyzed using Two-way ANOVA with Student-Newman Keuls tests ($p<0.05$). Results: Morphological and chemical analyses of the material surfaces were performed by using ESEM-EDX after 28d in HBSS. MTA Repair HP showed superior radiopacity to that of MTA Vitalcem and similar to MTA Angelus. All materials showed a marked alkalinizing activity within 3h, which continued for 28d. MTA Repair HP showed the highest calcium release at 28d ($p<0.05$). MTA Vitalcem showed statistically higher water sorption and solubility values ($p<0.05$). Conclusions: All the materials showed the ability to nucleate calcium phosphate on their surface after 28d in HBSS. MTA Repair HP and MTA Vitalcem had some positives properties, such as extended alkalinizing activity and calcium release that favored calcium phosphate nucleation. However, MTA Vitalcem showed scant radiopacity, long setting time and high solubility in comparison with the other tested materials. MTA Repair HP showed similar results with MTA Angelus but presence of the plasticizer might increased its solubility and porosity.

Keywords: Physical Properties. Materials testing. Endodontics. Calcium Silicate. Dental Materials.

INTRODUCTION

The chemical-physical properties of calcium silicate-based cements, such as ion release, solubility, porosity, setting time and radiopacity are of the utmost importance as far as their clinical usefulness is concerned. Their good biological properties are attributed to their capacity for alkaline pH and calcium ion release[4,21]. In addition, the capacity to spontaneously produce a calcium phosphate apatite-like layer on their surface when in contact with phosphate-containing fluids is largely attributed to calcium ion release and maintenance of a high pH for a long period of time[21, 11,12].

Conventional Mineral trioxide aggregate (MTA) cements are calcium silicate-based materials mainly composed of Portland cement, with the addition of bismuth oxide as a radiopacifier[3]. It has been shown that even small chemical differences[11,7] , or inclusion of additives in low percentages[2,25,4,7] , or changes in the radiopacifying agent[24,8,21] may strongly modify the chemical-physical behavior of these materials.

Recently, new formulations have been introduced to the market[7,25,9,24,8]. Among them, MTA Repair HP and MTA Vitalcem have been proposed.

MTA Repair HP is based on the formulation of MTA Angelus but contains Calcium Tungstate as radiopacifier and a new mixing liquid with a plasticizer agent. It is proposed for use as root-end filling, pulp capping, pulpotomy, apexogenesis, apexification and to repair root canal perforations. According to the manufacturer's instructions, this new formula maintains the chemical properties of the original MTA Angelus, but changes its physical properties related to manipulation. No further information is available and no studies have been published.

MTA Vitalcem cement has a composition similar to that of conventional MTA[5] but contains Zirconium Dioxide as radiopacifier. MTA Vitalcem has been proposed use as for root-end filling, perforation repair, root resorption, apexification, and pulp capping. It has shown antimicrobial properties[20] and regenerating properties[5] similar to those of MTA Angelus.

In the literature up to now, there are no studies on the chemical-physical characterization of these new cements.

Thus, the aim of the present study was to investigate the calcium release, pH, solubility, porosity, water sorption, radiopacity, and calcium phosphate nucleation in simulated body fluid of MTA Repair HP and MTA Vitalcem.

MATERIALS AND METHODS

Materials

The materials used in the study were the MTA Repair HP, MTA Vitalcem and White MTA Angelus (control) (Table 1). All the materials were prepared according to the manufacturer's instructions.

Physical Properties: Radiopacity, Setting Time, Porosity, Solubility and Water Sorption

The radiopacity was analyzed by following the Gandolfi et al.[8] (2012) methodology, in accordance with ISO 6876:2002. Freshly mixed samples (10±0.1 mm diameter; 1.0±0.1 mm height) were radiographed using a radiographic unit (Myray Cefla, Imola, Italy) with an aluminum step wedge (60 mm long, 10 mm wide) as reference. The digitized radiographs were evaluated and the radiographic density values were converted into aluminum step-wedge equivalent thickness (mm Al) using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA).

To establish the setting time (n=3 for each material), the freshly mixed pastes were compacted into molds (10 mm diameter, 2 mm thickness) and then stored at 37°C and 95%±5% relative humidity. The final setting time was registered when no indentation was caused by a needle weighing 453.6 g, with a tip diameter of 1.06 mm.

For the porosity, another set of disks (8±0.1 mm diameter x 1.6±0.1 mm) (n=10 for each material) were set at 37ºC and 99% relative humidity for a period equal to 70% of their setting time[12,7] in compliance with ISO 6876. Demolded specimens were weighed to determine the initial mass (D_i) and immersed vertically in 20 mL of distilled water at 37 °C. After 24 hours, the mass while suspended in water (S) was determined and (after removing the excess water from the surface of each sample with moistened filter paper) the saturated mass (M) was recorded[12,7,9]. Samples were dried at 37ºC until the weight was stable (dry mass [D_f]). Each weight measurement was repeated 3 times to the nearest 0.001 g using an analytical balance (Bel Engineering series M, Monza, Italy).

The solubility ($S = [(I - D)/D] \times 100$) and the water sorption ($WS = [([M - D_f])/D_f] \times 100$) were calculated as percentage of the original weight[7,9,8]. The exterior volume V ($V = M - S$), open pore volume V_{OP} ($V_{OP} = M - D$), impervious portion volume V_{IP} ($V_{IP} = D_f - S$), and apparent porosity P ($P = [([M - D_f])/V] \times 100$) were calculated by

Archimedes' principle by following the Gandolfi et al. [7,9,10] (2015, 2014, 2015) method.

Alkalizing Activity (pH of Soaking Water) and Calcium Release

The test was performed as described by Gandolfi et al[9] (2014). The molds (8±0.1 mm diameter x 1.6±0.1 mm, n=10 for each material) were immersed in 10 mL of deionized water at 37ºC[12,7,9]. The soaking water was collected and replaced after time intervals of 3 and 24 h, and 7, 14, 28 days[7,9]. Calcium ions (ppm) and alkalinizing activity (pH) of the soaking water were analyzed by placing the beaker containing it on a magnetic stirrer, and using a multiparameter laboratory meter (inoLab 750 WTW, Weilheim, Germany) connected to a calcium probe (Calcium ion electrode; Eutech instruments Pte Ldt, Singapore) or a (selective) temperature-compensated pH probe/electrode (Sen Tix Sur WTW, Weilheim, Germany). For the Calcium analysis, the solution was supplemented with 200 µL (2%) ionic strength adjuster (4 mol/L KCl, WTW, Weilheim, Germany).

Calcium phosphate nucleation

Freshly mixed samples (8±0.1 mm diameter x 1.6±0.1 mm) were immersed vertically in 20 mL of HBSS (Hank's Balanced Salt Solution, Lonza Walkersville, Inc., Walkersville, MD, USA) by following the Gandolfi et al. method[11,7] and stored at 37°C for 28 days. The HBSS was renewed weekly. Fresh samples and 28 days old samples were examined using an environmental scanning electron microscope (ESEM; Zeiss EVO 50, Carl Zeiss, Oberkochen, Germany) connected to an energy dispersive x-ray (EDX; Oxford Instruments, Abingdon, UK)[15]. EDX data were used to calculate the surface calcium-to-phosphorus (Ca/P) atomic ratios[11,7,9,10].

Statistical Analysis

The normally distributed data were analyzed by parametric testing (Sigma Stat, San Jose, CA, USA) by using two-way ANOVA followed by the RM Student-Newman-Keuls test (statistically significant difference for $p < 0.05$).

RESULTS

Physical Properties

Radiopacity, final setting time, solubility, porosity and water sorption are summarized in Table 2.

MTA Repair HP showed similar radiopacity to that of MTA Angelus with no statistical difference ($p>0.05$), whereas MTA Vitalcem presented the lowest radiopacity.

MTA Repair HP showed similar final setting time values to those of MTA Angelus with no statistical difference ($p>0.05$). MTA Vitalcem presented markedly higher values that differed statistically from both those of MTA Repair HP and MTA Angelus.

MTA Angelus showed the lowest values of solubility, open pore volume, apparent porosity and water sorption; while MTA Vitalcem had the highest values of water sorption and solubility. MTA Repair HP and MTA Vitalcem showed similar open pore volume and apparent porosity values. However, MTA Repair HP had significantly lower solubility and water sorption values when compared with MTA Vitalcem.

Ion Release

The alkalinizing activity and calcium release values are summarized in Table 3 (sections A and B).

All materials produced an elevated pH during the first 7 days, subsequently the decrease in their alkalinizing activity was statistically significant. At 14 days, MTA Angelus produced a statistically significant higher pH whilst at 28 days the alkalinizing activity was similar for all materials with no statistical difference ($p>0.05$).

The calcium release decreased with time for all the materials. The calcium ion release after 3 hours was markedly higher for MTA Vitalcem in comparison with MTA Angelus and MTA Repair HP; however from 1d up to 28d, the MTA Repair HP calcium release was the highest and statistically higher than that of both MTA Angelus and MTA Vitalcem. The Ca release of MTA Repair HP was the most stable and considerable among the materials. Ca release diminished for all materials as from 14 days.

Calcium phosphate nucleation

The ESEM/EDX results of freshly prepared and 28 days old samples with the qualitative semi-quantitative elemental composition are shown in Figure 1.

The Ca/P deposits differing in amount and size were detected by ESEM on the surface of the materials after 28 days immersion in HBSS.

Freshly mixed MTA Angelus cement showed high calcium (Ca) (34.34% wt) and silicon (Si) (6.06% wt) content, and traces of aluminum (Al) (1.78% wt). Well evident elongated particles of bismuth were visible. Following 28 days in HBSS the surface was coated with irregularly distributed Ca (28.61% wt) and P (10.79% wt) precipitates (Ca/P= 2.05) and Bi was not detectable.

Freshly mixed MTA Repair HP showed a uniform surface containing interspersed granules of tungsten (W) (11.72% wt), and displayed Ca (19.84% wt) and Si (2.68% wt). After 28d-soaking in HBSS, the surface was covered with globular precipitates, the Si component disappeared and Na (0.90% wt), Mg (1.13% wt), and P (14.83% wt) elements from HBSS became detectable.

Freshly mixed MTA Vitalcem displayed a granular surface showing mainly Ca (13.16% wt) and Si (6.78% wt). Al (1.42% wt), Zr (6.19% wt) and S (1.16% wt), were noted. After 28 days in HBSS, the surface was coated with globular precipitates. P (10.38% wt) element appeared and amounts of Na (0.22% wt), K (0.17% wt) and Mg (1.11% wt) were also noted. Zr, Al and S components became undetectable.

DISCUSSION

In the present study, two new calcium silicate MTA based cements, each containing a different radiopacifying agent alternative to bismuth oxide were compared with MTA Angelus relative to their physical properties, ion release and ability to nucleate calcium phosphate on their surface.

Bismuth oxide has been the component responsible for the color alteration of MTA and consequently tooth discoloration[28,27,19]. Reports that bismuth oxide interfered with MTA hydration and caused deterioration in the mechanical properties[3], have encouraged research on the substitution of bismuth oxide by other radiopacifiers. Zirconium oxide and calcium tungstate are the radiopacifier of MTA Vitalcem and MTA Repair HP, respectively. Marciano et al.[19] (2014) revealed that none of these radiopacifiers induced cement discoloration in contact with collagen. Duarte et al.[16] (2009) showed that both radiopacifiers associated with Portland cement in a ratio of 20% met the ISO 6876 requirements (radiopacity $\geq$3 mm Al), displaying a radiopacity of 3.41 mm Al for the cement with Zirconium Oxide and 3.11 mm Al with Calcium Tungstate[16]. Another study comparing MTA Angelus with these radiopacifiers associated with PC (in the same ratio as was proposed by the previous study) showed that all materials promoted calcium ion release and an alkaline pH, therefore, the authors considered Calcium Tungstate and Zirconium oxide potential radiopacifying agents to be used in combination with PC[17]. In our results, MTA Repair HP and MTA Angelus presented similar radiopacity values (5.81 and 4.50 mm Al, respectively) meeting the requirements of ISO 6876 while the radiopacity of MTA Vitalcem (2.46 mm Al) did not reach the recommended value.

In endodontic surgery, cements with long setting times are more susceptible to wash-out and dissolution[12]. For this reason laboratory studies assessing the setting times and the solubility of endodontic cements may be of interest to clinicians. In our study, the final setting time of MTA Angelus was about 84 min, in agreement with previous studies[21,18]. MTA Repair HP showed a similar final setting time (85 min) without statistical differences when compared with MTA Angelus. The setting time of MTA Vitalcem resulted the longest (140 min) time, with statistical differences; the long setting time may make this cement more susceptible to dissolution.

The solubility of calcium silicate-based cements is related to the formation of soluble calcium salts and calcium hydroxide during the hydration and to the setting reactions of the material[7]. In the present study MTA Angelus showed a solubility of 4.91%, which is in agreement with a study that reported a solubility of 3.47%, however, a different methodology was used[29]. Another study that used the same methodology as ours, displayed a higher solubility (29.55%)[13]. These differences could be correlated with changes in the formulation, as highlighted by the EDX data. MTA Repair HP showed a statistically higher solubility (8.18%) compared with that of MTA Angelus. This difference between MTA Angelus and MTA Repair HP, which have similar compositions, could be due to the plasticizer contained in the mixing liquid of MTA Repair HP. From our laboratory experience, the plasticizer (included - according to the manufacturer’s brochure - to facilitate its manipulation and insertion into the root cavity) seemed to improve the manipulation of MTA Repair HP compared with MTA Angelus (4.91%), however, as a result the material was more soluble. In Addition, Cintra et al.[30] (2017) showed that MTA Repair HP has a good cellular viability, which could be related to the solubility of this material. The solubility of MTA Vitalcem (14.19%) was markedly higher than that of the other materials.

In the present study, all the materials showed alkalinizing activity and calcium release. These properties of calcium silicate cements are due to both the formation of calcium hydroxide and the release of calcium from the calcium silicate particles[21,15,30]. The materials presented similar alkalinizing activity results from 3h to 7d; statistically significant higher values were obtained for MTA Angelus at 14d. MTA Angelus showed similar results compared with those of other studies[11,29,23]. The decrease in alkalinizing activity with time, observed in our results, was also shown by Prati and Gandolfi[21] (2015). Other studies have found lower values of pH in all periods[17,18].

The release of calcium is a key factor for successful pulp capping therapy perforation repair, apexification, root resorption and many other endodontic procedures because of the action of calcium on pulp cell differentiation and hard tissue mineralization[21,22,26]. In our study, MTA Vitalcem showed the highest initial (3h) calcium ion release (143.1 ppm) decreasing with time until 14d when it presented the lowest release (7.58 ppm) compared with all the tested materials. MTA Repair HP showed the highest release ($p<0.05$) values from 1d to 28d. MTA Angelus showed the highest calcium release at 7d (50.64 ppm), presenting similar results to those of other studies[21,7]. A recent study, with similar evaluation time intervals as ours, found lower calcium release values [18].

MTA Vitalcem showed the highest water sorption value (18.14%); this result could be correlated with its highest values of calcium release, final setting time, and open and apparent porosity (31.37%). For MTA Repair HP, the high water sorption (14.96%) could be correlated to the presence of the plasticizing agent. MTA Angelus showed the statistically significant lower values of water sorption (12.04%) and apparent porosity (25.02%).

The growth of a calcium phosphate (apatite) layer creates an ideal environment for stem cell and osteoblast differentiation and colonization to support new bone formation[21,12,9]. ESEM/EDX analysis provided qualitative and semi-quantitative measurements of atomic calcium and phosphorous to calculate the superficial calcium to phosphorus (Ca/P) atomic ratios[11,14,23]. In our results, all the materials showed the ability to nucleate calcium phosphate on their surface and the Ca/P atomic ratio at 28 days was 2.05 for MTA Angelus, 1.63 for MTA Repair HP and 1.87 for MTA Vitalcem. The lower Ca/P atomic ratio of MTA Repair HP could be correlated to the presence of the plasticizer.

CONCLUSION

In summary, the study showed that the novel cements MTA Repair HP and MTA Vitalcem had some positive properties, particularly the extended alkalinizing activity and calcium release which favored calcium phosphate nucleation. However, MTA Vitalcem showed scant radiopacity, a long setting time and high solubility in comparison with the other tested materials. MTA Repair HP showed similar results with MTA Angelus, but the presence of the plasticizer may perhaps have increased the solubility and porosity. The radiopacifier Calcium Tungstate can be used to replace Bismuth Oxide.

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FIGURE LEGENDS

Figure 1– ESEM/EDX of freshly prepared and 28 days old samples with the qualitative semi-quantitative elemental composition of the materials.

Table 1. Composition of the materials used

Materials (and manufacturers)	Lot number (and expiration date)	Composition	Mixing Ratio
MTA Vitalcem (Catholic University of Santa María, Arequipa, Peru)	110014 (2016-10)	**Powder:** CaO , ZrO_2 , Fe_2O_3 , MgO , Alumina , Hydrated Silica , K_2O , Na_2O **Suggested (non provided) liquid:** Distillated Water	1 g of powder 0,3 g of liquid
MTA Repair HP (Angelus, Londrina, Brazil)	34263 (2020-03)	**Powder:** $3CaO.SiO_2$, $2CaO.SiO_2$, 3CaO. Al_2O_3 , CaO , $CaWO_4$ **Liquid:** H_2O and Plasticizer	0,085 g of powder (1 package) 0,25 g of liquid (1 drop)
MTA Angelus (Angelus, Londrina, Brazil)	30317 (2017-02)	**Powder:** SiO_2 , K_2O , Al_2O_3 , Na_2O , Fe_2O_3 , SO_3 , CaO , Bi_2O_3 , MgO , insoluble residues of CaO , KSO_4 , $NaSO_4$ and crystalline silica. **Liquid:** Distillated Water	0,145 g of powder (1 spoon) 0,04 g of liquid (1 drop)

Table 2. Radiopacity, final setting time, solubility, porosity and water sorption of the tested materials. Different small letters represent statistically significant differences (two-way ANOVA followed by Student-Newman-Keuls with $p<0.05$) in the same column.

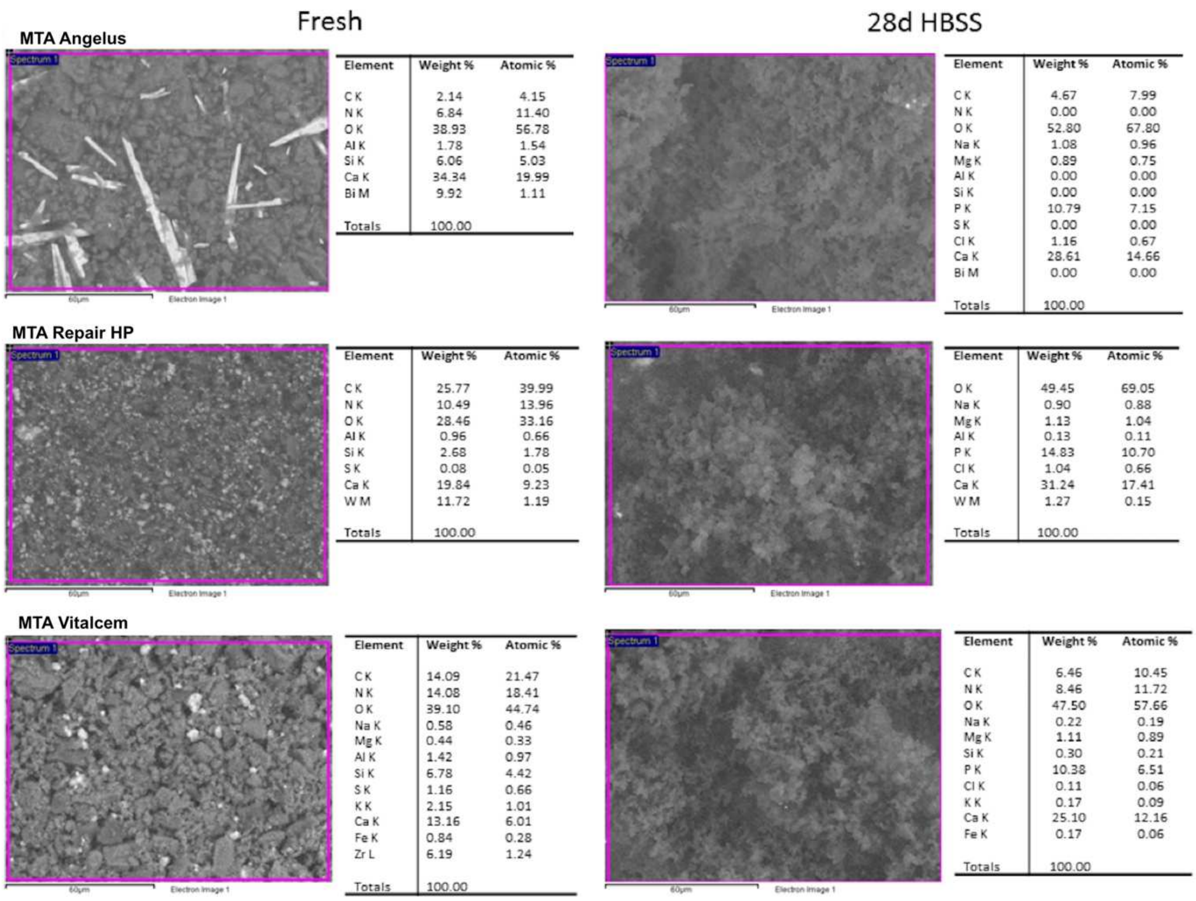
	Radiopacity (mm Al)	Final Setting Time (m)	Solubility (%)	Exterior Volume (cm^3)	Volume of Open Pores (cm^3)	Volume of Impervious Portion (cm^3)	Apparent Porosity (Vop/V %)	Water sorption (%)
MTA Vitalcem	2.46±0.17 [b]	140±2 [a]	14.19±1.32 [a]	0.0913±0.0056 [a]	0.0286±0.0010 [b]	0.0627±0.0049 [a]	31.37±1.38 [a]	18.14±1.07 [a]
MTA Repair HP	4.50±0.46 [a]	85±2.64 [b]	3.18±1.74 [b]	0.0877±0.0045 [a]	0.0258±0.0006 [b]	0.0619±0.0044 [a]	29.45±1.49 [a]	14.96±0.95 [b]
MTA Angelus	5.81±0.55 [a]	34.33±5.13 [b]	4.91±3.73 [c]	0.0873±0.0015 [a]	0.0218±0.0033 [a]	0.0655±0.0037 [a]	25.02±3.89 [b]	12.04±2.60 [c]

Table 3 – (A) pH of soaking water; (B) Calcium ions released in soaking water. Different capital letters represent statistically significance differences ($p<0.05$) in the same line, whilst different small letters represent differences in the same column.

(A) **pH of soaking water**	3 hours	1 day	3 days	7 days	14 days	28 days
MTA Vitalcem	11.54 ± 0.09^{Aa}	11.51 ± 0.54^{Aa}	11.15 ± 0.30^{Aa}	11.11 ± 0.28^{Aa}	9.13 ± 0.60^{Bb}	9.59 ± 0.48^{Ca}
MTA Repair HP	11.37 ± 0.06^{Aa}	11.30 ± 0.09^{Aa}	11.03 ± 0.10^{Aa}	11.24 ± 0.47^{Aa}	9.23 ± 0.47^{Bb}	9.05 ± 0.35^{Ba}
MTA Angelus	11.34 ± 0.31^{Aa}	11.28 ± 0.60^{Aa}	11.08 ± 0.63^{Aa}	11.20 ± 0.44^{Aa}	10.31 ± 0.51^{Ba}	9.13 ± 0.49^{Ca}
distilled water	6.7 ± 0.3^{Ab}	6.8 ± 0.1^{Ab}	7.2 ± 0.4^{Ab}	7.1 ± 0.1^{Ab}	6.5 ± 0.6^{Ac}	7.2 ± 0.5^{Ab}

(B) **Calcium released (ppm)**	3 hours	1 day	3 days	7 days	14 days	28 days
MTA Vitalcem	143.1 ± 7.10^{Aa}	45.11 ± 6.35^{Bb}	35.85 ± 4.32^{Ca}	24.79 ± 3.90^{Db}	7.58 ± 1.18^{Fc}	11.85 ± 0.92^{Eb}
MTA Repair HP	48.81 ± 3.24^{Bb}	60.77 ± 4.80^{Aa}	40.32 ± 3.54^{Ca}	51.04 ± 4.83^{Ba}	21.02 ± 2.11^{Da}	14.80 ± 1.58^{Ea}
MTA Angelus	49.65 ± 6.97^{Ab}	33.77 ± 7.82^{Bc}	23.91 ± 6.08^{Cb}	50.64 ± 2.63^{Aa}	17.47 ± 4.87^{Db}	11.53 ± 1.25^{Eb}
distilled water	1.0 ± 0.8^{Ac}	0.6 ± 1.1^{Ad}	1.8 ± 0.7^{Ac}	1.6 ± 0.8^{Ac}	1.2 ± 0.9^{Ad}	1.3 ± 0.3^{Ac}

Figure 1



MTA Angelus – Fresh

Element	Weight %	Atomic %
C K	2.14	4.15
N K	6.84	11.40
O K	38.93	56.78
Al K	1.78	1.54
Si K	6.06	5.03
Ca K	34.34	19.99
Bi M	9.92	1.11
Totals	100.00	

MTA Angelus – 28d HBSS

Element	Weight %	Atomic %
C K	4.67	7.99
N K	0.00	0.00
O K	52.80	67.80
Na K	1.08	0.96
Mg K	0.89	0.75
Al K	0.00	0.00
Si K	0.00	0.00
P K	10.79	7.15
S K	0.00	0.00
Cl K	1.16	0.67
Ca K	28.61	14.66
Bi M	0.00	0.00
Totals	100.00	

MTA Repair HP – Fresh

Element	Weight %	Atomic %
C K	25.77	39.99
N K	10.49	13.96
O K	28.46	33.16
Al K	0.96	0.66
Si K	2.68	1.78
S K	0.08	0.05
Ca K	19.84	9.23
W M	11.72	1.19
Totals	100.00	

MTA Repair HP – 28d HBSS

Element	Weight %	Atomic %
O K	49.45	69.05
Na K	0.90	0.88
Mg K	1.13	1.04
Al K	0.13	0.11
P K	14.83	10.70
Cl K	1.04	0.66
Ca K	31.24	17.41
W M	1.27	0.15
Totals	100.00	

MTA Vitalcem – Fresh

Element	Weight %	Atomic %
C K	14.09	21.47
N K	14.08	18.41
O K	39.10	44.74
Na K	0.58	0.46
Mg K	0.44	0.33
Al K	1.42	0.97
Si K	6.78	4.42
S K	1.16	0.66
K K	2.15	1.01
Ca K	13.16	6.01
Fe K	0.84	0.28
Zr L	6.19	1.24
Totals	100.00	

MTA Vitalcem – 28d HBSS

Element	Weight %	Atomic %
C K	6.46	10.45
N K	8.46	11.72
O K	47.50	57.66
Na K	0.22	0.19
Mg K	1.11	0.89
Si K	0.30	0.21
P K	10.38	6.51
Cl K	0.11	0.06
K K	0.17	0.09
Ca K	25.10	12.16
Fe K	0.17	0.06
Totals	100.00	

2.3 Article 3 - Chemical-physical properties and apatite-forming ability of MTA Flow.

The accepted article presented in this Thesis was written according to the Journal of Endodontics *instructions and guidelines for article submission*

ABSTRACT

Introduction: This study aimed to analyze the following chemical-physical properties: pH, volumetric change, radiopacity and apatite-forming ability in simulated body fluid (SBF) of a new tricalcium silicate material (MTA Flow, Ultradent Products Inc, South Jordan, UT, USA).

Methods: MTA Flow was tested in comparison with MTA Angelus. The pH of soaking water was tested up to 168h in deionized water. In the solubility test, the root-end fillings of 20 acrylic teeth were scanned twice by a Micro-CT, before and after immersion in ultrapure water for 168 h. In addition, using an aluminum step-wedge, the radiopacity of each material was evaluated as recommended by international standards. Mean gray values of the test materials were measured using Image J software. The morphological and chemical analyses of the material surface were performed by using SEM-EDX after 28d in HBSS. The data were analyzed by using Two-way ANOVA with Student-Newman Keuls test ($p<0.05$).

Results: MTA Flow showed similar alkalizing activity to that of MTA Angelus. In the solubility test both materials presented lower values without statistical differences. Both materials showed a marked alkalinizing activity within 3h, which continued for 168h. MTA Angelus showed statistically higher radiopacity values ($p<0.05$). All materials showed the ability to nucleate calcium phosphate on their surface after 28d in HBSS.

***Conclusions*:** MTA Flow showed remarkable alkalinizing capability, low solubility, good radiopacity and ability to form calcium phosphate deposits after being soaked in simulated body fluid, showing values similar to those of MTA Angelus.

INTRODUCTION

Tricalcium silicate materials, such as the well-known MTA (Mineral Trioxide Aggregate), are biomaterials widely used for reparative procedures in dentistry and endodontics (1). Primarily composed of tricalcium silicates, MTA cements are radiopaque materials that form a self-setting calcium-silicate-hydrate gel when manipulated with water (2).

The first formulation of MTA, patented in 1995, was composed of grey Portland cement (PC) with addition of bismuth oxide powder as a radiopacifier (3,4). In 2004, Primus (5) successively proposed the white version of MTA constituted of white PC without iron in its composition and also using bismuth oxide as a radiopacifier. MTA's performance is mostly attributable to its bioactivity; that is, the ability to produce an apatite layer when in contact with physiological fluids (6,7).

Some properties of MTA cements, such as sealing ability (8–10) correlated to expansion (11); the capacity to set in the presence of fluids; and they hydroxyl and calcium ion release which provides an alkaline pH unfavourable for bacterial growth and formation of a mineralized barrier in adjacent tissues into the surrounding environment (12–14), seem to be appropriate for their clinical use. Thus, new tricalcium silicate materials have been introduced on market.

MTA Flow^{TM} (Ultradent Products Inc, South Jordan, UT, USA) is a novel MTA-like cement composed of a grey powder of di- and tricalcium silicate that sets with a water-based gel. It is proposed for use in root-end filling, as a dressing over pulpotomies, pulp capping, perforation repair, apexification, root resorption and many other endodontic procedures.

According to the manufacturer, the new gel-based vehicle makes the cement more washout-resistant and additionally gives the professionals a variety of consistency that can be adapted to every procedure. In the literature at present, there are no studies on the chemical-physical characterization of this new cement.

Thus, the aim of the present study was to investigate the chemico-physical properties, namely pH, volumetric change, radiopacity and apatite-forming ability in simulated body fluid (SBF) of MTA Flow in comparison with MTA Angelus (Angelus, Londrina, PR, Brazil). The null hypothesis was that there wouldn't be difference between MTA Flow and control.

MATERIALS AND METHODS

Materials

The materials used in the study were MTA Flow and MTA Angelus. Samples of all the materials were prepared in accordance with the respective manufacturer's instructions. MTA Angelus was prepared using 3:1 powder to liquid ratio. MTA Flow was prepared in a putty consistency using 0.19g of powder with one drop of gel.

Alkalizing Activity (pH)

The above-mentioned samples (8±0.1 mm diameter x 1.6±0.1 mm, n=10 for each material) were immersed in 10 mL of distilled water at 37ºC. The soaking water was collected and replaced after time intervals of 3, 24, 72 and 168h. Alkalinizing activity (pH) of the soaking water was measured with a pH meter (model 371; Micronal, São Paulo, SP, Brazil), previously calibrated with buffer solutions of pH 4, 7 and 14. After removing the specimens from the container, it was placed in a shaker (model 251; Farmem, São Paulo, SP, Brazil) for 5s before the pH was measured, at a room temperature of 25°C.

Volumetric change

Twenty acrylic teeth (n = 10) with a standardized root-end cavity with 3-mm depth were used. The cavities were filled with the cements and the samples were immediately scanned with a desktop X-ray microfocus CT scanner (SkyScan 1174v2; SkyScan, Kontich, Belgium). The scanning procedure was completed by using 50 kV X-ray tube voltages, 800 lA anode current. Digital data were elaborated by reconstruction software (NRecon- onv1.6.4.8, SkyScan), and the CTan software (CTan v1.11.10.0, SkyScan) was used for the volume measurements. After the scanning procedure and elapse of the final setting time, the samples was individually immersed in glass flasks containing 15 mL of ultrapure water, and then stored at 37 °C for 168 h. Subsequently, the samples were removed from the flasks, dried with filter paper and then scanned again by using exactly the same parameters adopted for the first scan. The volume change was determined in percentage by calculating the volume of cement that was lost during immersion, as reported previously by Cavenago et al. (15).

Radiopacity

The radiopacity was analyzed in accordance with the ISO 6876:2012 standard. Three cylindrical samples (10±0.1 mm diameter; 1.0±0.1 mm height) were prepared (as previously described), and radiographed on occlusal films (D-speed; Kodak Comp, Rochester, NY) with a reference aluminum step wedge (60 mm long, 10 mm wide) by using a radiographic unit (Gnatus XR 6010; Gnatus, Ribeirão Preto, SP, Brazil). The radiographs were digitized using a desktop scanner (SnapScan 1236; Agfa, Germany) and the radiographic density values were evaluated and converted into aluminum step-wedge equivalent thickness (mm Al) by means of ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Apatite-forming ability

Molds filled with freshly prepared cement (8±0.1 mm diameter x 1.6±0.1 mm) were vertically immersed in 20 mL of HBSS (Hank's Balanced Salt Solution, Lonza Walkersville, Inc., Walkersville, MD, USA) and stored at 37°C for 28 days. The HBSS was renewed every week. Fresh samples and 28d-aged samples were examined using an elemental analysis by energy-dispersive X-ray spectroscopy (EDX) that was fully integrated into the Aspex Express Scanning Electron Microscope (SEM) (Fei Europe, Eindhoven, The Netherlands). EDX data were used to calculate the surface calcium-to-phosphorus (Ca/P) atomic ratios.

Statistical Analysis

Data were submitted to normality test of D'Agostino & Pearson. Two-way repeated measures analysis of variance statistical analysis was performed followed by the Student-Newman Keuls test ($P < 0.05$).

RESULTS

Alkalizing Activity (pH), Volumetric change and Radiopacity

The alkalinizing activity, volumetric change and Radiopacity values are summarized in Table 1.

Both cements induced alkalization of the soaking water that reduced with time, but was still present in the final periods analyzed. At 186 hours, the pH level of the materials was significantly reduced in comparison with the initial time intervals of 3 and 24h. Overall, the alkalinizing activity was similar for both cements with no statistical difference between them for all periods ($p>0.05$).

MTA Flow showed similar volume change to MTA Angelus with no statistical difference ($p>0.05$), whereas, for radiopacity, MTA flow presented a significantly lower value.

Apatite-forming ability

The ESEM/EDX results of freshly prepared and 28d-aged samples with the qualitative, semi-quantitative, elemental composition of MTA Flow and MTA Angelus are shown in Figures 1 and 2, respectively. Calcium phosphate deposits were detected by SEM on the surface of the materials after 28 days of immersion in SBF.

Fresh MTA Flow presented an uniform surface. Elongated particles of bismuth oxide with variable sizes were evident in cement matrix. Peaks of calcium (Ca) (36.10% wt); silicon (Si) (10.40% wt); bismuth (Bi) (8.30% wt), and aluminum (Al) (4.60% wt) were also detected in EDX. After 28 days in HBSS, the peak of Ca increased, whereas all other elemental peaks diminished. The surface was coated with irregularly distributed Ca (37.80% wt) and P (10.30% wt) precipitates and Bi was not detectable.

Freshly mixed MTA Angelus displayed an irregular surface with evident granules, and displayed EDX reflexes of Ca (37.60% wt); Si (9.60% wt); Bi (10.50% wt); and Al (4.10% wt) similar to MTA Flow. Elongated particles mainly consisting of bismuth interspersed with cement particles could be observed. After 28 days in HBSS, the phosphorous (P) (6.50% wt) element; traces of sodium (Na) (6.10% wt) and chlorine Cl (6.90% wt) from the NaCl present in the HBSS were eminent. The surface was coated with irregularly distributed Ca (22.10% wt) and P (6.50% wt) precipitates. The component Si decreased in intensity and no S and Bi was detected. The Ca/P atomic ratio was 2.63.

DISCUSSION

The present study tested a material recently introduced on the market, containing an alternative vehicle to distilled water (MTA Flow^{TM}, Ultradent Products Inc, South Jordan, UT, USA) together with a gold standard tricalcium silicate formulation (MTA Angelus, Londrina, PR, Brazil); and compared their alkalizing activity, physical properties, and ability to nucleate calcium phosphate on their surface. The null hypothesis was disproved, since there was difference between MTA Flow and MTA Angelus properties in the radiopacity test.

The characteristics of manipulation of MTA Angelus have been frequently discussed and several authors proposed alteration of liquid to improve the flowability of this cement and facilitate insertion (16-18). The use of anti-washout gel was also proposed with the aim to increase viscosity and resistance of particles to external water solutions (19). The anti-washout gel enhances physical properties such as compressive strength, reduces setting time and porosity, but also interfere in hydration (19). MTA Flow is composed of a water-based gel vehicle and a powder of tricalcium and dicalcium silicate. From our laboratory experience, the water-based gel seemed to facilitate its manipulation and insertion in cavity when compared with MTA Angelus.

MTA cements are known to have high pH, resulting from the hydration process (20). High pH levels are usually verified in the initial periods, before complete setting (21). The capacity to release hydroxide ions stimulates the release of alkaline phosphatase and bone morphogenetic protein 2, which participate in the mineralization process (22,23). Several studies have demonstrated that MTA-based materials stimulates hard tissue repair (24,25). The cements presented similar alkalinizing activity results from 3h to 168h, showing no statistically significance between them ($p<0.05$). MTA Angelus showed similar results compared with other studies (26,27). The decreased in alkalinizing activity with the material aging, observed in our results, has also been shown in other studies (26,28).

The volume loss of a root-end filling cement is undesirable because this defect reduces its sealing ability, thereby creating the conditions for bacterial and fluid microleakage, compromising the treatment (29). The ISO 6876:2012 recommends the test to evaluate the solubility of materials. According to this standard method, the initial and final weights after immersion are considered to calculate the solubility (30). In this study, micro-CT volumetric data was used to evaluate the MTA volume change (15). In this innovative methodology, the clinical condition is reproduced allowing that a reasonable surface of cement be exposed to the immersion solution. The volumetric analysis also prevents the water sorption as a variable (31). The volume change presented by MTA Angelus was low (1.25) and similar to that verified in the study of Cavenago et al. (2014) (15) (1.83) and Duque et al. (2017) (32) (1.93).

The radiopacity of tricalcium silicate cements associated with different radiopacifying agents has been evaluated by other authors (33,34). Among the tested radiopacifiers, bismuth oxide (Bi_2O_3) revealed the highest radiopacity. This explained why Bi_2O_3 has been widely used in many MTA materials. It is known that MTA Angelus has Bi_2O_3 in its formulation (33), but the MTA Flow manufacturer did not disclose its radiopacifier. However, by means of our SEM/EDX test we showed that Bi_2O_3 was also the radiopacifying agent used in this cement. The radiopacity evaluation showed that MTA Angelus had a higher radiopaque value than MTA Flow ($p<0.05$) although both materials presented radiopacity higher than 3mm Al, as recommended by ISO 6876:2012 specification (30). MTA Angelus showed results similar to those of another study (35).

Both cements presented the ability to form calcium phosphate deposits after being soaked in SBF. These deposits provide clinical advantages by creating an excellent environment for stem cell and osteoblast differentiation; colonization; and for supporting new bone formation (36). Moreover, their deposition over time improved sealing at the interface between the tooth and material (37). ESEM/EDX analysis provided qualitative and semi-quantitative measurements of atomic calcium and phosphorous that allowed calculation of the superficial calcium to phosphorus (Ca/P) atomic ratios. The high intensity of peaks for Ca and P detected in EDX analysis was indicative of precipitation of amorphous deposits corresponding to calcium phosphate (38). The high Ca/P atomic ratios presented at 28 days immersion in SBF, both for MTA Flow and MTA Angelus (2.05 and 1.63, respectively), could indicate this deposition on the cement surface. This amorphous calcium phosphate precipitation (precursor of apatite) is considered a bioactive characteristic of cements (38). Further XDR analysis would precisely indicate the crystalline composition of the structures (39). The Ca/P ratios of MTA Flow were higher in comparison to MTA Angelus. This difference could be related to the anti-washout gel vehicle, which could enhance some chemical properties of the cement as pointed by Formosa et al. (2013) (19).

In summary the study revealed that the novel cement MTA Flow showed alkalinizing capability, low solubility, good radiopacity and ability to form calcium phosphate deposits after being soaked in SBF, with values similar to those of MTA Angelus. For clinicians, MTA Flow represents a new bioactive calcium silicate material, and therefore seems to be an alternative to the conventional MTA cements, nevertheless, further research is necessary.

ACKNOWLEDGMENTS

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The authors deny any conflicts of interest related to this study.

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FIGURE LEGENDS

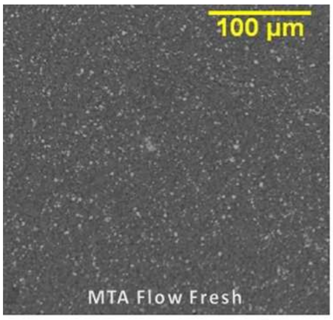
Figure 1: SEM images with EDX microanalysis evaluating the chemical composition of MTA Flow, freshly prepared and soaked for 28 days in HBSS.

Figure 2: SEM images with EDX microanalysis evaluating the chemical composition of MTA Angelus, freshly prepared and soaked for 28 days in HBSS.

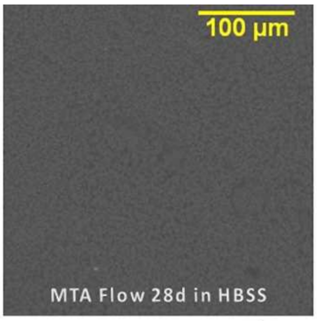
Table 1. pH of soaking water, Volumetric change and Radiopacity. Different capital letters represent statistically significance differences ($p<0.05$) in the same column, whilst different small letters represent differences in the same line.

	pH of soaking water				Volumetric Solubility (%)	Radiopacity (mm Al)
	3 hours	24 hours	72 hours	168 hours		
MTA Flow	10.1 ± 0.87^{Aa}	9.7 ± 0.54^{Aa}	9.3 ± 0.78^{Aab}	8.5 ± 0.82^{Ab}	1.28 ± 0.76^{A}	4.8 ± 0.43^{A}
MTA Angelus	9.8 ± 0.75^{Aa}	9.3 ± 0.53^{Aac}	9.0 ± 0.45^{Abc}	8.7 ± 0.80^{Ab}	1.25 ± 0.69^{A}	6.9 ± 0.35^{B}

FIGURE 1



Element	Weight%	Atomic%
O K	40,60	63,14
Al K	4,60	4,24
Si K	10,40	9,21
Ca K	36,10	22,41
Bi M	8,30	0,99
Totals	100.00	



Element	Weight%	Atomic%
O K	43,10	62,83
Na K	2,80	2,84
Al K	0,00	0,00
Si K	3,60	2,99
P K	10,30	7,76
Cl K	2,40	1,58
Ca K	37,80	22,00
Bi M	0,00	0,00
Totals	100.00	

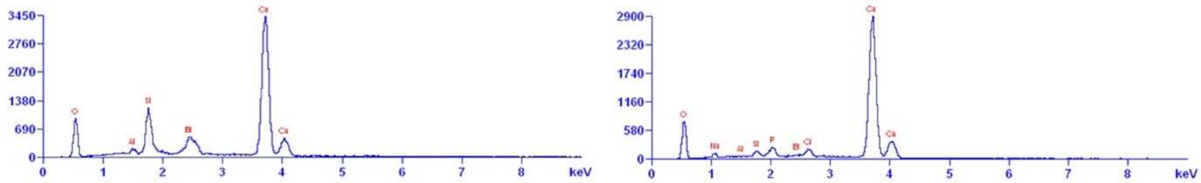
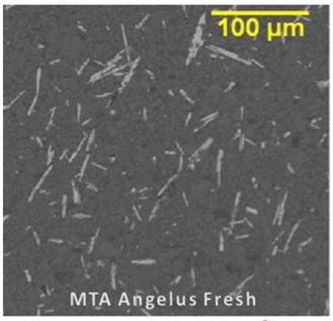
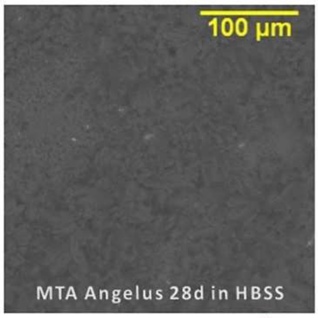


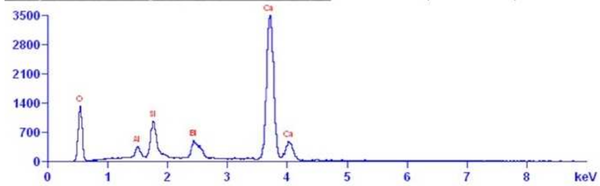
FIGURE 2

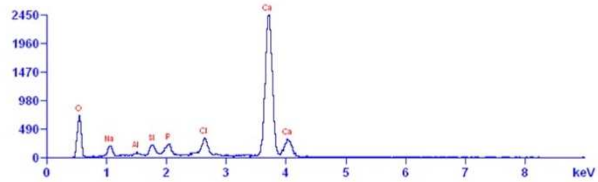


Element	Weight%	Atomic%
O K	38,20	61,70
Al K	4,10	3,93
Si K	9,60	8,83
Ca K	37,60	24,24
Bi M	10,50	1,30
Totals	100.00	



Element	Weight%	Atomic%
O K	50,70	67,87
Na K	6,10	5,68
Al K	3,50	2,78
Si K	4,20	3,20
P K	6,50	4,49
Cl K	6,90	4,17
Ca K	22,10	11,81
Bi M	0,00	0,00
Totals	100.00	





3 DISCUSSION

3 DISCUSSION

MTA-based materials are used in contact with periapical tissue or with vital pulp. For this reason, they should have specific biological properties like biocompatibility, biointeractivity (release of biologically relevant ions), and bioactivity (apatite-forming ability) in order to promote the activity of mineralizing cells and the formation of new periapical bone or reparative dentine. Moreover, color is one of the most important factors to be considered in esthetic dentistry. The initial formulation of MTA was a gray powder and was related with coronal tooth discoloration (BOGEN et al., 2008; KARABUCAK et al., 2005). For that reason, the white version of MTA was developed and has been commercially available since 2002 (ASGARY et al., 2004). Although, it is also reported that white MTA promoted tooth discoloration after pulpotomy (BELOBROV & PARASHOS, 2011) but when MTA was removed, significant color change of the dental crown was observed.

Despite that many factors can contribute to tooth discoloration in the presence of MTA (CAMILLERI, 2014; VALLÉS et al., 2013a, 2013b), the contamination with blood has been investigated in a number of laboratory studies in terms of the effect on its physical properties, leakage, displacement, marginal adaptation and colour alterations. The mechanisms of how blood contamination exacerbates the discoloration process and how we can minimize this alteration was analyzed in the first part of this study. The vehicle composed of 80% DW/20% PG was proposed to improve MTA mixing and to analyze the influence on cement color change.

Initially, the red blood cells were studied. The structure called porphyrin, is a large heterocyclic organic ring, made up of four pyrrolic groups joined together by methine bridges. A ferrous (Fe^{2+}) ion can be found in the center of porphyrin molecule. In our study, we showed by using SEM/EDX analysis that this molecule is basically composed of C (carbon), N (nitrogen) and O (oxygen). In addition, the SEM/EDX analysis suggested that the porosities of MTA may uptake blood components. This information was also observed in the study of Lenherr et al. (LENHERR et al., 2012). The natural redox reaction causing the loss Fe^{2+} (present in the center of porphyrin molecule) and becomes ferric (Fe^{3+}), which possesses a dark brown color, may result in a darkening of the cement.

The bismuth oxide, radiopacifier present in MTA Angelus, is related to color alteration of MTA (CAMILLERI, 2014; FELMAN & PARASHOS, 2013; MARCIANO et al., 2014). In our first study, the decrease of radiopacity caused by the dissociation of the radiopacifier was observed and was similarly reported by other authors (CAMILLERI, 2008; CAVENAGO et al., 2014) corroborating with our color assessment results. The ratio of 80% DW/20% PG used as vehicle of MTA can improved flowability, pH, and calcium ion release as showed by Duarte et al. (DUARTE et al., 2012) and can be recommend in cases where esthetics are crucial and blood contamination is likely since its use presented lower color alteration.

The second and third part of this study focused on analyse some recent material introduced in market together with a popular calcium silicate-based formulation (MTA Angelus, Angelus, Londrina, PR, Brazil) comparing their physical-chemical properties and the bio-properties of biointeractivity and bioactivity. MTA Repair HP (Angelus, Londrina, PR, Brazil) and MTA Vitalcem (School of Dentistry, Catholic University of Santa María, Peru) was compared in our second part of this study.

MTA Repair HP is a recent calcium silicate-based cement material that was developed to improve the performance of the traditional MTA Angelus. The powder is composed mainly of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium oxide, calcium carbonate and calcium tungstate (radiopacifier) (SILVA et al., 2016) . According to the manufacturer, the high-plasticity and improved physical properties are due the replacement of the distilled water by a liquid containing water and a plasticizer agent. Recently, Silva et al. showed that MTA Repair HP presented better push-out bond strength when compared with its predecessor MTA Angelus (SILVA et al., 2016). Another recent study by Cintra et al. showed that its biocompatibility and biomineralization are similar to MTA-Angelus presenting also an increased fibroblast cell viability compared after a longer period (CINTRA et al., 2017). In our study the physical-chemical properties and bioactivity was evaluated. MTA Repair HP presented in its formulation a replacement of the bismuth oxide radiopacifier by a calcium tungstate radiopacifier with the intuition of avoid dental discoloration as showed by a previous study (MARCIANO et al., 2014). Our results showed that MTA Repair HP has similar radiopacity values with its predecessor (5.81mm Al) meeting the requirements of ISO 6876. Duarte et al. found that when associated with Portland cement, the calcium tungstate also met the ISO 6876 requirements (radiopacity ≥3 mm Al) (HÚNGARO DUARTE et al., 2009).

In relation to alkalinizing activity and calcium release, MTA Repair HP showed some similar properties when compared with MTA Angelus. MTA Repair HP showed the highest calcium release ($p<0.05$) values from 1d to 28d. This result is probably due the presence of calcium tungstate as a radiopacifier agent in MTA Repair HP contributing to the higher calcium release (HUNGARO DUARTE et al., 2012) and consequently promoting greater biomineralization corroborating with our bioactivity test. The presence of the plasticizer, which the composition is not disclosed by the manufacturer, may perhaps have increased the solubility and porosity of the material.

MTA Vitalcem is another new calcium silicate-based cement from Peru. It has a composition similar to that of conventional MTA Angelus but contains Zirconium Dioxide as radiopacifier (FIGUEROA & OBANDO, 2014). The cement is composed of a grey powder of calcium silicate that sets with distilled water. From our laboratory experience the manipulation with distilled water resulted in a sandy and dry material. Zirconium oxide as a radiopacifier presented good radiopacity results when associated in a ratio of 20% with Portland cement (HÚNGARO DUARTE et al., 2009) and not induced cement discoloration when in contact with collagen (MARCIANO et al., 2014). Although, in our results, MTA Vitalcem did not reach the radiopacity requirements of ISO 6876 presenting 2.46 mm Al. This is probably occurred because of a deficiency in the quantity of the radiopacifier.

MTA Vitalcem presented a good alkalinizing activity and calcium release. It showed the highest initial (3h) calcium ion release (143.1 ppm). This extended calcium release could be associated with the high solubility that was found in our results (14.19%). The highest water sorption value (18.14%) presented by MTA Vitalcem can be correlated also to its high values of initial calcium release. In summary, all the materials analyzed in this second part of the showed the ability to nucleate calcium phosphate on their surface. The radiopacifier Calcium Tungstate seems suitable to replace Bismuth Oxide.

In the third part of this study, some physical-chemical properties and bioactivity of the new MTA cement from Ultradent called MTA Flow (Ultradent Products Inc, South

Jordan, UT, USA) was analyzed in comparison to MTA Angelus. One of the main feature of this cement, according to the manufacturer, is that the mixing ratio is adaptable to every procedure, allowing the clinician to achieve any desired consistency. In addition, according to the manufacturer, the water-based gel vehicle gives an effective, anti-washout, and easy-to-deliver MTA. Formosa et al. showed that anti-washout gel vehicle enhances physical properties such as compressive strength, reduces setting time and porosity, but also interfere in hydration (FORMOSA et al., 2013). MTA Flow is composed of a grey powder of di- and tricalcium silicate. In our analysis made by means of our SEM/EDX test we showed that bismuth oxide is the radiopacifying agent used in MTA Flow.

Hydroxide ions stimulate the release of alkaline phosphatase and BMP-2, which participate in the mineralization process (MAEDA et al., 2010; MOGHADDAME-JAFARI et al., 2005). In our results, the alkalinizing activity of MTA Flow presented similar to MTA Angelus from 3h to 168h, showing no statistically significance between them ($p<0.05$).

The solubility of a root-end filling cement is undesirable because such defects reduce their sealing ability creating the conditions for bacterial and fluid microleakage, compromising the treatment (WU et al., 1995). Microcomputed tomography (micro-CT) is a nondestructive 3D imaging technique used, in the endodontic field, to evaluate root canal anatomy (MILANEZI DE ALMEIDA et al., 2013; VERMA & LOVE, 2011), root canal geometry and shapes (HÜBSCHER et al., 2003) or to estimate root canal morphology and the spatial details after root canal instrumentation (MARKVART et al., 2012). In this study, a methodology to evaluate the MTA solubility (volume change) after root-end filling procedures using micro-CT volumetric data was used (CAVENAGO et al., 2014). In this innovative methodology, the clinical condition is reproduced allowing that a reasonable surface of cement be exposed to the immersion solution. In our study, MTA Flow showed similar volume change compared with MTA Angelus (1.28 and 1.25 respectively) with no statistical difference ($p>0.05$). Similar volume change results for MTA Angelus was found in a recent study (DUQUE et al., 2017).

It is know that bismuth oxide is the radiopacifier of MTA Angelus (HÚNGARO DUARTE et al., 2009). Although both materials presented the same radiopacifier, our result showed that MTA Angelus is significantly more radiopaque ($p<0.05$). Additionally, it is important to notify that both materials presented radiopacity higher than 3mm Al, as recommended by ISO 6876:2012 specification.

The apatite-forming ability (bioactivity) together with the ion release may well explain the excellent clinical outcomes of MTA cements (PARIROKH & TORABINEJAD, 2010b). The growth of a layer of apatite is an ideal environment for stem cell and osteoblasts differentiation and colonization, and to support new bone formation (LAURENT et al., 2008). The amorphous calcium phosphate precipitation (precursor of apatite) is considered a bioactive characteristic of cements (MOINZADEH et al., 2016). After 28 days of immersion in simulated body fluid (SBF), both MTA Flow and MTA Angelus cements presented high Ca/P atomic ratios indicating deposition of calcium phosphate.

In summary, the third study revealed that the novel cement MTA Flow showed alkalinizing capability, low solubility, good radiopacity and ability to form calcium phosphate deposits after being soaked in SBF, with values similar to those of MTA Angelus.

4 CONCLUSIONS

4 CONCLUSIONS

- Although MTA discoloration has been associated with its radiopacifier, i.e., bismuth, and the oxidation of the heme group present in hemoglobin can exacerbate this discoloration. The ratio of 80% DW/20% PG as a vehicle results in lower color alteration when in contact with blood. This can be recommend in cases where esthetics are crucial and blood contamination is likely, such as coronal perforations, pulp capping, and coronal sealing in revascularization, to achieve clinically acceptable levels of color alteration.

- The novel cements MTA Repair HP and MTA Vitalcem had some positive properties, particularly the extended alkalinizing activity and calcium release which favored calcium phosphate nucleation. However, MTA Vitalcem showed scant radiopacity, a long setting time and high solubility. MTA Repair HP showed good and prolonged calcium release and the ability to nucleate an apatite-like layer, but the presence of the plasticizer may perhaps have increased the solubility and porosity. The radiopacifier Calcium Tungstate can be used to replace Bismuth Oxide.

- The novel cement MTA Flow showed alkalinizing capability, low solubility, good radiopacity and ability to form calcium phosphate deposits after being soaked in SBF, with values similar to those of MTA Angelus. For clinicians, MTA Flow represents a new bioactive calcium silicate material, and therefore seems to be an alternative to the conventional MTA cements, nevertheless, further research is necessary.

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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 ***Color Stability, Radiopacity, and Chemical Characteristics of White Mineral Trioxide Aggregate Associated with 2 Different Vehicles in Contact with Blood*** will be included in the Thesis of the student (Bruno Martini Guimarães) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, May 22th 2017.

Bruno Martini Guimarães
Author

Bruno Martini Guimarães
Signature

Talita Tartari
Author

Signature

Marina Angélica Marciano
Author

Marina A. M.
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Rodrigo Ricci Vivan
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Rafael Francisco Lia Mondeli
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Josette Camilleri
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Marco Antonio Hungaro Duarte
Author

Marco A H Duarte
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APENDIX B - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article ***Novel calcium silicate-based formulations (MTA Repair HP and MTA Vitalcem) with new radiopacifiers*** will be included in the Thesis of the student (Bruno Martini Guimarães) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, May 22th 2017.

Bruno Martini Guimarães
Author

Signature

Carlo Prati
Author

Signature

Marco Antonio Hungaro Duarte
Author

Signature

Clovis Monteiro Bramante
Author

Signature

Maria Giovanna Gandolfi
Author

Signature

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

We hereby declare that we are aware of the article ***Chemical-physical properties and apatite-forming ability of MTA Flow*** will be included in the Thesis of the student (Bruno Martini Guimarães) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, May 22th 2017.

Bruno Martini Guimarães
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ANNEXES

ANNEX A – Permission letter to include a published article from The Journal of Endodontics in this thesis

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13-Mar-2017

Dear Dr. Guimarães:

Your manuscript entitled "Novel calcium silicate-based formulations (MTA Repair HP and MTA Vitalcem) with new radiopacifiers" has been successfully submitted online and is presently being given full consideration for publication in the Journal of Applied Oral Science.

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ANNEX C – Article acceptance letter from The Journal of Endodontics

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Ref.: Ms. No. JOE 17-174R1
Chemical-physical properties and apatite-forming ability of MTA Flow

Dear Dr. Guimarães,

I am pleased to inform you that your manuscript has now been accepted for publication in Journal of Endodontics.

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Thank you for submitting this manuscript. I look forward to seeing it published soon.

With kind regards,

Ken Hargreaves
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