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Physical, chemical, and biological properties of white MTA with additions of AlF3

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Clinical Oral Investigations

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--Manuscript Draft--

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Funding Information:	FAPESP (2014/01003-6, 2017/05096-7) — Dr. Marina Angélica Marciano
Abstract:	Objectives Addition of aluminium fluoride (AlF3) to MTA was tested to inhibit dental discolouration. Materials and methods MTA Angelus with 0, 5, 15 and 45% AlF3 were tested. The set cements were characterized using scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction. Radiopacity and setting time were analysed according to ANSI/ADA 57 and ASTM C266-08. Volume change was evaluated using volumetric micro-CT analysis. The pH and calcium ion release were assessed after 3h, 24h, and 28d. Dental discolouration in contact with the cements was assessed after 24h, 28d and 90d of contact with bovine and human dentine. Tissue reaction to subcutaneous implantation in rats was examined after 30 and 60d. Results AlF3 altered the microstructure of MTA. The addition of 5% AlF3 did not significantly alter the radiopacity, setting time and volume change ($p > 0.05$). pH and calcium ion release significantly increased with addition of AlF3 ($p > 0.05$). All the tested proportions of AlF3 prevented the dental darkening verified for MTA Angelus in bovine and human teeth. AlF3 did not interfere in inflammatory response of MTA in all periods of analysis, otherwise lower amounts showed less intense inflammatory infiltrate. Conclusion The use of 5% of AlF3 in combination to MTA resulted in a cement that did not result in dental discolouration and did not affect significantly physical, chemical and biological properties. Clinical significance AlF3 prevents destabilization of bismuth oxide and consequent tooth darkening, frequently verified in clinical practice when using white MTA.
Response to Reviewers:	1. Concern of the reviewer: Not only contact of the MTA with dentin, but also with blood or sodium hypochlorite causes discoloration. Will the addition of aluminium chloride prevent discoloration equally well? Can this be concluded on the basis of the experiments in teeth included in this study?

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Revised text: In the study, only the interaction between material and dentine was tested. The literature shows that other factors can be associated with discolouration such as the contact with sodium hypochlorite or blood [11, 25]. It is possible that the addition of aluminum fluoride also prevents the discolouration of MTA caused by sodium hypochlorite. A similar reaction is verified in the interaction bismuth oxide/sodium hypochlorite and bismuth oxide/collagen of dentine [11]. The phase change of bismuth oxide leads to dark precipitate. In the presence of blood, another component is related with this reaction, that is the iron released from hemoglobin [25]. Further investigations can demonstrate the ability of aluminum fluoride to prevent discolouration of MTA in different conditions.

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(a) Buccal surface of bovine tooth (b) Pulp chamber surface of bovine tooth with the cavity prepared.

7. Why the triple antibiotic was used as a positive control? And how was it possible to keep it in place without dissolution after 90 day storage in tab water? And why is it tab water and not deionized or saline?

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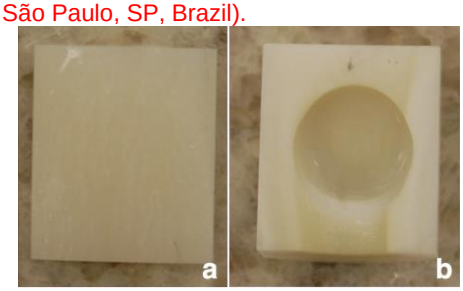

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Physical, chemical and biological properties of white MTA with additions of AlF_3

Marina Angélica Marciano[1], Josette Camilleri[2], Ribamar Lazanha Lucateli[3], Reginaldo Mendonça Costa[4], Mariza Akemi Matsumoto[5], Marco Antonio Hungaro Duarte[4]

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[2]Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Malta.

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[4]Department of Dentistry, Endodontics and Dental Materials, Dental School of Bauru, University of São Paulo - USP, Bauru, SP, Brazil.

[5]Department of Morphology, Dental School of Araçatuba, State University of São Paulo - UNESP, Araçatuba, SP, Brazil.

Running head: Aluminium fluoride to prevent discolouration of MTA

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Abstract

Objectives Addition of aluminium fluoride (AlF_3) to MTA was tested to inhibit dental discolouration.

Materials and methods MTA Angelus with 0, 5, 15 and 45% AlF_3 were tested. The set cements were characterized using scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction. Radiopacity and setting time were analysed according to ANSI/ADA 57 and ASTM C266-08. Volume change was evaluated using volumetric micro-CT analysis. The pH and calcium ion release were assessed after 3h, 24h, and 28d. Dental discolouration in contact with the cements was assessed after 24h, 28d and 90d of contact with bovine and human dentine. Tissue reaction to subcutaneous implantation in rats was examined after 30 and 60d.

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Conclusions The use of 5% of AlF_3 in combination to MTA resulted in a cement that did not result in dental discolouration and did not affect significantly physical, chemical and biological properties.

Clinical relevance AlF_3 prevents destabilization of bismuth oxide and consequent tooth darkening, frequently verified in clinical practice when using white MTA.

Key words: Cement; Biomaterials; Biocompatibility; Colour.

Introduction

Colour is an important property in aesthetic dentistry. Mineral trioxide aggregate (MTA) is a tricalcium silicate-based cement indicated for several purposes including apexifications,

apical surgery, pulp capping and perforation repair [1, 2]. The first formulation of MTA was gray, which limited its use in anterior teeth [2, 3]. An white MTA has reduced amounts of FeO in comparison with gray formulation [3]. Although white MTA had been introduced to eliminate tooth discolouration, several studies verified that this cement darkens in teeth over time [3-9].

White MTA includes bismuth oxide to enhance the material radiopacity [10]. The literature has shown that bismuth oxide is involved in tooth discolouration. The destabilization of bismuth molecules by certain factors, ie, amino acids of dentine, contact with sodium hypochlorite and the combination of oxygen-free environment and irradiation with cure light, was identified [9-12]. A phase change from its oxide form may occur resulting in dark precipitate in dentine [13]. The application of bonding agent to obliterate dentine tubules was suggested by Akbari *et al.* (2012) [3] to minimize long-term discolouration. This procedure requires an additional step that prolongs the treatment and depends on the complete sealing to be efficient. Another alternative is the substitution of the radiopacifier [14, 15]. Previous studies demonstrated that larger amounts of some radiopacifiers, such as zirconia, are required to provide similar radiopacity of bismuth oxide [14, 16, 17].

The addition of substances that prevents destabilization of bismuth oxide and consequent tooth discolouration can be an alternative [13]. The aim of the current study was to investigate the colour alteration of white MTA with additions of aluminium fluoride and its effect on physical, chemical and biological properties.

Materials and methods

The materials used in this study included white MTA Angelus (Angelus, Londrina, Paraná, Brazil) as the control and white MTA Angelus with additions of 5, 15 or 45% aluminium fluoride (AlF_3 - Sigma-Aldrich, St. Louis, MO, USA). The aluminium fluoride was dosed by weight. All cements were mixed using distilled water at a powder to liquid ratio of 0.3 (1g powder to 0.3 mL liquid).

Characterization of materials

Microscopy and elemental analysis

Cylindrical specimens 10 mm in diameter and 2 mm high were prepared and stored in HBSS for 28 days at 37°C. At the end of the storage period the specimens were taken out of solution, dried, vacuum desiccated and embedded in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark). The specimens were polished with progressively finer diamond discs and pastes using an automatic polishing machine (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens were attached to aluminium stubs, carbon coated and viewed with a scanning electron microscope (SEM; Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs of the material were recorded in back-scatter electron mode and energy dispersive spectroscopy (EDS) was carried out.

X-ray Diffraction (XRD) analysis

Disc-shaped specimens 15 mm in diameter and 2 mm high were prepared and allowed to set at 37ºC and 95% ± 5% relative humidity for 24 hours. They were immersed in HBSS for 28 days after which they were retrieved, dried and crushed to a fine powder using a mortar and pestle. Phase analysis was performed with a Bruker D8 diffractometer (Bruker Corp., Billerica, MA, USA) with Co Kα radiation (1.78A˚). The X-ray patterns were acquired in the 2θ (15–45°) with a step of 0.02° and 0.6 seconds per step using the Bragg Brentano method. Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

Radiopacity

Three disk-shaped samples 10 mm in diameter and 1 mm in thickness of each cement type were prepared. The disks were maintained at 37°C during 24 hours for complete setting of the materials. The specimen thickness was checked with a digital caliper (Mitutoyo Corp, Tokyo, Japan). If required, the specimens were ground wet with 600-grit silicon carbide paper (Buehler Ltd., Lake Bluff, IL) to reach the standardized thickness. The samples were radiographed on occlusal films (D-speed; Kodak Comp, Rochester, NY) with an aluminium step-wedge graduated from 2 to 16 mm in thickness. A radiographic unit (Gnatus XR 6010; Gnatus, Ribeirão Preto, SP, Brazil) was used with exposures set at 60 kVp, 10mA, 0.3 seconds and a focus-film distance of 30 cm. The radiographs were digitized and analyzed using Digora 1.51 software (Soredex, Helsinki, Finland). The radiopacity was determined as previously described [14].

Setting time

The setting time was determined according to the American Society for Testing and Materials specifications (ASTM C266-08), but the samples were made following the ISO (ISO 6876:2012) standard. The cements were mixed and placed into stainless steel rings with a 10 mm internal diameter and 2 mm in height. Three stainless steel rings were filled with each material and stored in an incubator at 37ºC ± 1ºC and 95% ± 5% relative humidity. Then, a 113.4 g Gilmore needle was used for determination of the initial setting time. The final setting time was determined with a 453.6 g Gilmore needle. This procedure was repeated at 60-s intervals. The setting times were considered as the moment at which the needle did not leave a complete circular indentation on the surface of the specimen.

Volume change

The volume change test was performed using volumetric micro-CT measurements [18]. Forty acrylic teeth (n = 10) with a standardized root-end cavity were used. The cavities were filled with the freshly mixed cement and the samples were scanned using a desktop X-ray microfocus CT scanner (SkyScan 1174v2; SkyScan, Kontich, Belgium). The scanning was performed using 50 kV X-ray tube voltages, 800 µA anode current. Four samples were scanned at a time. The image capture parameters used were of a voxel size of 14.1 µm with 1.1° rotation step using a 360° rotation. Each scan consisted of 327 TIFF images with 1024 x 1304 pixels. Digital data were further elaborated by reconstruction software (NReconv1.6.4.8, SkyScan), and the CTan software (CTan v1.11.10.0, SkyScan) was utilized for the volume measurements. In the CTan software, the total volume of cement was calculated in mm^3. After scanning, the samples were individually immersed in flasks containing 15 mL of ultrapure water and stored at 37ºC for 28 days. After the period of immersion, the samples were dried and scanned again. The volume change was determined in percentage by calculating the volume of cement that was lost during immersion.

pH and calcium ion release in solution

Forty acrylic teeth (n=10) with a cavity of 3-mm depth were filled with the different materials and immersed individually in 10 mL of deionized water and stored at 37 °C. After 3 h, 24 h and 28 days, the teeth were placed in new flasks containing an equal volume of new deionized water. The pH of the water in which the teeth had been kept was measured with a pH meter (model 371; Micronal, São Paulo, SP, Brazil), previously calibrated using buffer solutions of pH 4, 7 and 14.

For determination of calcium ion release, an atomic absorption spectrophotometer was used (AA6800; Schimadzu, Tokyo, Japan). This reading was performed in the same periods used for the pH level measurement.

Assessment of tooth colour

Sample preparation

A total of 20 human teeth and 20 bovine teeth were prepared. For human teeth, the crowns were vertically sectioned with a 0.3 mm diamond disc (Isomet, Buehler, Lake Bluff, Illinois, USA). Bovine teeth were sectioned in the crown in 10 × 10 mm blocks. A 3 mm diameter cavity and 1.5 mm deep was prepared in the center of pulp chamber surface of human and bovine teeth with a high-speed diamond bur 4054 (Medical Burs Sorensen, São Paulo, SP, Brazil). The specimens were washed with distilled water and dried with gauze. The external limit of the cavities was conditioned with 37% phosphoric acid for 30 seconds, washed with distilled water for 1 minute, and dried with an air syringe. A layer of adhesive (Adper Single Bond 2; 3M ESPE, Sumaré, SP, Brazil) was applied to the conditioned external limit of the cavity and light-cured (Optilight LD Max; Gnatus, Ribeirão Preto, SP, Brazil) for 20 seconds to allow the sealing of the interface with resin. The cements were compacted into the prepared cavities at a depth of 1.5 mm. Triple antibiotic paste was used as positive control and unfilled specimens were used as negative control as previously described [12]. After the cements set, the cavities were sealed with a natural flow resin B2 (Nova DFL, Rio de Janeiro, RJ, Brazil). The polymerization was performed with an LED curing light (Optilight LD Max) for 60 seconds. The specimens were stored separated in dark flasks and were immersed in tap water at 37° C throughout the period of analysis (90 days).

Spectrophotometry

The colour assessments were performed: immediately after filling, 24 hours, 28 days and 90 days after filling. The colour assessments were performed with a spectrophotometer (Vita Easyshade, VITA Zahnfabrik, Bad Sackingen, Germany). The assessments were performed in an ambient light. Excess water on the specimen was removed with gauze and the colour measured. The values of CIE (Commission Internationale de l'éclairage) [19] L*, a* and b* was recorded, and the colour change (ΔE) corresponding to the intervals was

calculated using the formula: $\Delta E = [(L_1-L_0)^2 + (a_1-a_0)^2 + (b_1-b_0)^2]^{1/2}$. The values of colour change (ΔE) and lightness (L) were considered to evaluate the darkening.

Stereomicroscopy

Representative samples of the materials were selected and horizontally sectioned in the center of the cavity using a 0.3-mm diamond disk. Polished longitudinal sections of material and tooth sections were viewed under the stereomicroscope (Stemi 2000C; Carl Zeiss, Jena, Germany) at 2× magnification. The images were acquired in software Axiovision (Carl Zeiss).

Tissue reaction

Sixteen adult male albino rats (*Rattus norvegicus*), weighing approximately 300 g were selected (Ethical approval CEP 014-2014). The study accomplished Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines. The mixed cements were inserted in sterile polyethylene tubes (10 mm in length and 1 mm in internal diameter) and immediately implanted subcutaneously in the dorsal region of the rats. The animals were divided into 4 groups (n = 4), according to the cement type. Each animal received 2 implants. For the surgical procedures, the rats were anesthetized with a combination of ketamine and xilasin (Vet Brands Int, Miramar, Florida).

After the experimental periods (30 and 60 days), the animals were sacrificed with a lethal dose of anesthetic. Sections of 5-µm thickness were stained with hematoxylin and eosin. Four sections from each specimen were selected. Histological evaluations were made under a light microscope (Olympus, São Paulo, Brazil) at 400x magnification, by a pathologist. For a quantitative evaluation of the inflammatory infiltrate, 30 microscopic fields were analyzed [20]: Grade 0 – Without inflammatory cells; Grade 1 – Sporadic presence of chronic inflammatory cells (< 25 cells); Grade 2 – Moderate infiltration of chronic inflammatory cells (25-125 cells); Grade 3 – Dense and severe infiltration of chronic

inflammatory cells (> 125 cells). The measurements were repeated twice to ensure reproducibility. The median and range of the grades were calculated.

Statistical analysis

Data were submitted to normality test of D'Agostino & Pearson. Statistical analysis was performed using ANOVA/Tukey tests for radiopacity, initial and final setting time, volume change, pH and calcium ion release and tissue reaction. The Kruskal-Wallis and Dunn's test were selected for analysis of discolouration ($p < 0.05$).

Results

Characterization

The scanning electron micrographs for all the cements are shown in Figure 1. The unmodified MTA Angelus exhibited a very well organized matrix with hydration product and some small unhydrated cement particles present in the matrix. The radiopacifier appeared whiter due to its higher atomic number. Peaks for bismuth and oxygen were shown on EDS analysis. The XRD scan of MTA Angelus exhibited a peak for calcium hydroxide (ICDD: 01-076-0571) at 18°2θ. The tricalcium silicate peaks 29°2θ, and bifid peak at ~32°2θ were flattened indicating the reduction of a crystalline phase and conversion to an amorphous phase which is not detected by X-ray diffraction. The presence of calcium hydroxide a reaction product indicates the degree of hydration of MTA Angelus in contact with HBSS for 28 days (Figure 2).

Addition of aluminium fluoride at 5% modified the cement hydration. The cement particles were intact with very little hydration (Figure 1) and the XRD scans had strong tricalcium silicate peaks at 29, 32 and 34°2θ indicating that little conversion to the amorphous calcium silicate hydrate state had occurred (Figure 2). The XRD scan of MTA Angelus showed none of these and only those for calcium hydroxide. No calcium hydroxide was formed in the 5% addition of aluminium fluoride. With increasing additions the microstructure changed and a structureless zone which had a high calcium peak was formed

(Figure 2). The XRD scans for the 15 and 45% addition of aluminium fluoride had no tricalcium silicate peaks at 29, 32 and 34°2θ indicating hydration. Crystalline calcium hydroxide was formed in the 15% AlF_3 containing samples as shown by the peak at 18°2θ, but not in the 45% aluminium fluoride addition. However, a Ca peak was noted in the EDX spectra (Figure 1). Aluminium fluoride (ICDD: 00-043-0435) as observed at ~25°2θ in the AlF_3 samples. Bismuth oxide (ICDD: 04-003-2034) having peaks at 26.90, 27.39, 28.01 and 33.0°2θ was present for all the materials.

Physical and chemical properties

The mean, standard deviation and statistical analysis of radiopacity, setting time and volume change are represented in Table 1. The cements showed statistically similar radiopacity (3-4 mm equivalent Al) ($p > 0.05$). For initial setting time, statistical difference was verified between 5% (14 min) and 15% AlF_3 (16 min) versus 0% AlF_3 (31 min) and 45% AlF_3 (33 min) ($p < 0.05$). The cements with 15 and 45% AlF_3 (95 and 107 min, respectively) had the longer setting times with statistical difference for the other groups ($p < 0.05$). The group 45% AlF_3 (2.24%) showed the higher volume change with statistical difference in comparison with 15% AlF_3 (0.76%) group ($p < 0.05$).

The results of pH and calcium ion release are shown in Table 2. All the materials had an alkaline pH (8-9 range). High pH values were maintained with 5 and 15% AlF_3 (9.8 and 9.7, respectively) for 28 days, statistically significant ($p < 0.05$). The cements containing aluminium fluoride had the higher calcium ion release in the final period of analysis (17-21 range), with statistical difference in comparison to MTA Angelus ($p < 0.05$).

Tooth colour

Mean, standard deviation, and statistical differences of the colour change (ΔE) and lightness (L) for bovine and human teeth in each period are shown in Table 1. Representative samples sectioned of each group are shown in Figure 3. The MTA without the AlF_3 had the

lowest lightness at 90 days ($p > 0.05$). The bovine and human lightness values were similar, with 10 units, but always higher for bovine samples ($p < 0.05$).

Biological properties

Representative tissue sections are shown in Figure 4. Median and range of inflammatory infiltrate are in Table 3. Microscopic analysis of specimens of each group revealed formation of connective tissue around the implanted tube in contact with cements. Predominance of lymphocytes cells was observed in the initial periods with decrease to the final period of analysis. In this period, the tissue showed capsular aspect. No statistical differences were verified in median and range among cements in all periods ($p > 0.05$). All samples had moderate inflammation or higher on average.

Discussion

Discolouration after contact with MTA has been previously reported [9, 11, 12]. The addition of 5, 15 and 45% aluminium fluoride to MTA Angelus inhibited discolouration of human and bovine teeth. Aluminium fluoride (AlF_3) is an inorganic compound, currently added to restorative materials due to its white colour and release of fluoride ions [21, 22]. A recent investigation showed similar results with additions of 5, 15 and 45% zinc oxide to MTA [13]. It is postulated that the molecules of bismuth oxide interacts with aluminum fluoride stabilizing it from phase changes [13]. The oxide form of bismuth is no longer altered, preventing dark precipitation on tooth.

The set materials were characterized by currently used techniques namely scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction [10]. This ensured complete characterization and determination of crystalline phases. In the current study, various proportions of aluminium fluoride were high (5, 15 and 45%) to test its effects on the properties of MTA Angelus. Aluminium fluoride altered the hydration of MTA, even with

small amounts. The cement particles react with water to initiate the hydration [10]. Thus, would be expected that the increase in the amount of aluminium fluoride results in more unreacted particles in cement matrix.

The addition of aluminium fluoride reduced the radiopacity by 30% radiopacity of MTA than 3 mm of Al, that is required by ANSI/ADA (57:2000) specification ($p > 0.05$). These results were similar to previous studies reported [15]. The addition of aluminium fluoride delayed the setting of the MTA Angelus with the 5% addition being least affected.

The volume change was assessed using a modified method. The solubility of test materials is usually tested in accordance to the ANSI/ADA 57:2000 or ISO 6876 guidelines. These methods are based on weights of cement samples before and after immersion [23]. The volumetric method proposed by Cavenago *et al.* (2014) [18] uses microtomography to determine the volume change. This methodology allows simulation of the clinical conditions with root-end cavities prepared in acrylic teeth [18]. Low changes in volume are expected to prevent gaps in interface or voids that could result in leakage [24]. The volume change of the tested cements was statistically similar to that shown for MTA Angelus ($p > 0.05$). The volume change for all materials tested in the current study was low and similar to values reported in a previous study [18].

Aluminium fluoride prevented tooth discolouration and this was demonstrated using both human and bovine model. The interaction between MTA Angelus and bovine or human tooth was previously demonstrated [8, 12]. The analysis in spectrophotometer based on CIE parameters [19] is a precise and quantitative method to assess colour [9, 11]. The reflectance of a surface can be detected by means of L (lightness), a (green-red axis) and b (blue-yellow axis) values, indicating the colour alteration [11]. A low value of L indicates that the surface darkened. In the study, the spectrophotometer analysis indicated that all cements promoted colour alteration, which was more intense for the MTA Angelus group. In the current study, the data of L indicated low values for MTA Angelus in comparison to the other materials tested, suggesting darkening of teeth filled with MTA Angelus. In stereomicroscopy, the discolouration of teeth was evident for the MTA Angelus group, with black staining

concentrated in the cement/dentine interface. The colour alteration was also seen in dental samples, suggesting that in clinical conditions MTA Angelus would compromise the aesthetics [5]. The minimum amount of aluminium fluoride tested was efficient to prevent tooth colour alteration, indicating that this substance can inhibit discolouration of MTA Angelus. Smaller proportions could be tested in further studies to indicate the minimal amount required to prevent colour alteration.

In the study, only the interaction between material and dentine was tested. The literature shows that other factors can be associated with discolouration such as the contact with sodium hypochlorite or blood [11, 25]. It is possible that the addition of aluminum fluoride also prevents the discolouration of MTA caused by sodium hypochlorite. A similar reaction is verified in the interaction bismuth oxide/sodium hypochlorite and bismuth oxide/collagen of dentine [11]. The phase change of bismuth oxide leads to dark precipitate. In the presence of blood, another component is related with this reaction, that is the iron released from hemoglobin [25]. Further investigations can demonstrate the ability of aluminum fluoride to prevent discolouration of MTA in different conditions.

The biological properties of the materials were assessed by subcutaneous implantation in experimental animals and these properties were correlated to the material chemical properties and degree of hydration. This has been used previously for dental materials testing [26, 27]. This methodology can be easily applied for initial analysis of biological properties *in vivo*. The results showed that the proportions of aluminium fluoride did not interfere in inflammatory response of MTA in all periods of analysis. High proportions of aluminium fluoride intensified the inflammatory infiltrate suggesting that lower amounts of these components are more adequate for biological properties of MTA.

Conclusion

The addition of 5, 15 and 45% aluminium fluoride to MTA Angelus inhibits dental discolouration. The 5% aluminium fluoride did not significantly influence the radiopacity, final setting time, volume change or subcutaneous reaction to MTA Angelus.

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Compliance with Ethical Standards

Conflict of Interest:

Author Marina Angélica Marciano declares that he has no conflict of interest.

Author Josette Camilleri declares that he has no conflict of interest.

Author Ribamar Lazanha Lucateli declares that he has no conflict of interest.

Author Reginaldo Mendonça Costa declares that he has no conflict of interest.

Author Mariza Akemi Matsumoto declares that he has no conflict of interest.

Author Marco Antonio Hungaro Duarte declares that he has no conflict of interest.

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent: For this type of study, formal consent is not required.

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Figure legends

Figure 1: Scanning electron micrographs and energy dispersive spectroscopic scans of MTA Angelus and modified MTA Angelus with additions of aluminium fluoride (AlF_3) in increasing proportions.

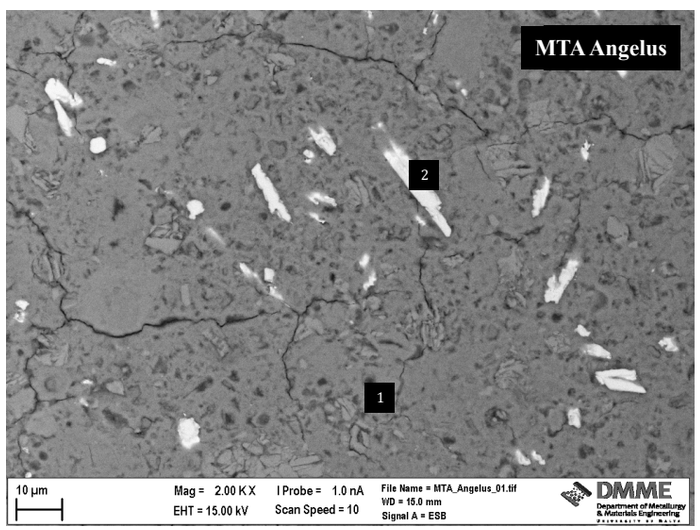
Figure 2: X-ray diffraction plots of MTA Angelus and MTA Angelus modified with different additions of aluminium fluoride (AF) showing peaks for the main crystalline phases present after immersion in Hank's balanced salt solution for 28 days (AF: aluminium fluoride: ICDD:00,043,0435; BO: bismuth oxide: ICDD: 04,003,2034; CH: calcium hydroxide: ICDD:01,076,0571; TCS: tricalcium silicate: ICDD: 00,049,0442).

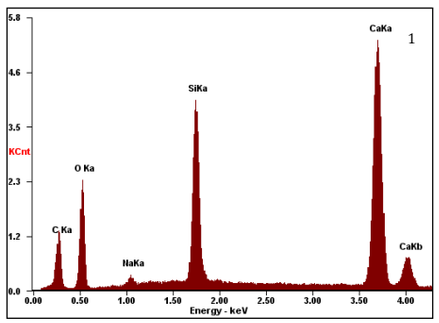
Figure 3: Representative samples sectioned of bovine and human tooth filled using MTA Angelus and MTA containing 5% of aluminium fluoride (5% AlF_3). The staining is evident in MTA Angelus group with colour alteration of the material and dentine. The groups in which aluminium fluoride was added did not showed colour alteration. Stereomicroscope images at × 2 magnification.

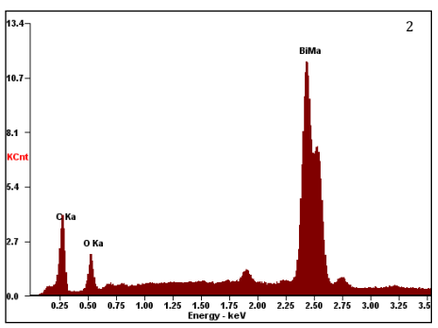
Figure 4: (a-d) Microscopic representative specimens of groups at 30 days of analysis. (a) MTA, (b) 5% AlF_3, (c) 15% AlF_3, (d) 45% AlF_3. Close to the material (#), inflammatory tissue (*) is present infiltrated by leucocytes. Areas with fibrous connective tissue in a capsule aspect (**) with slight infiltrate are observed. (e-h) Microscopic representative specimens at 60 days of analysis (e) MTA, (f) 5% AlF_3, (g) 15% AlF_3, (h) 45% AlF_3. At 60 days, is observed close to the material (#), inflammatory tissue (*) infiltrated by leucocytes. In some

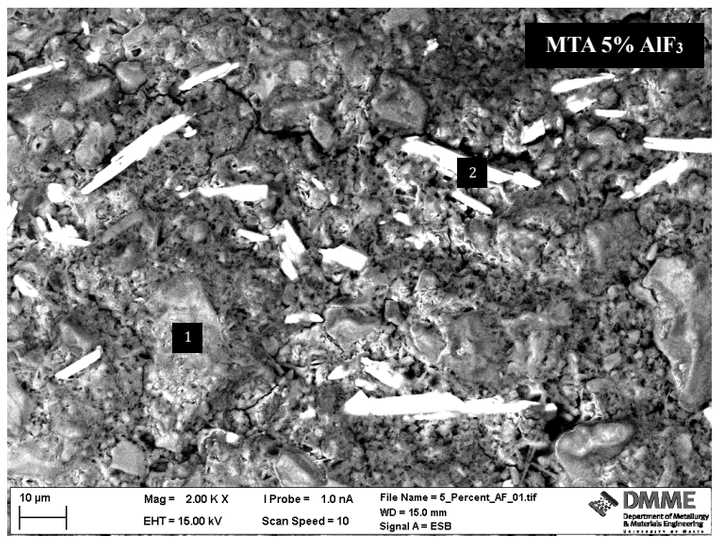
groups there was formation of fibrous capsule (**) with the presence of leucocytes. (HE; original magnification 20x).

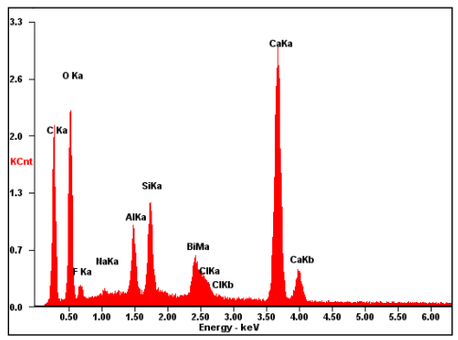
Figure 1

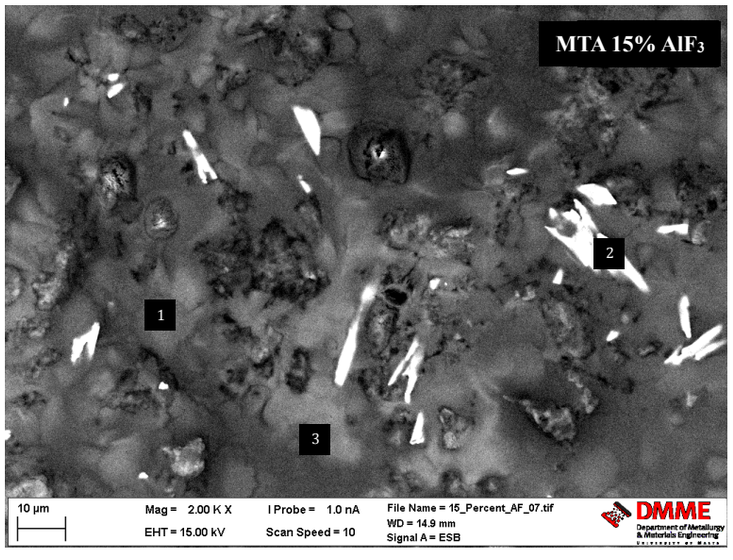


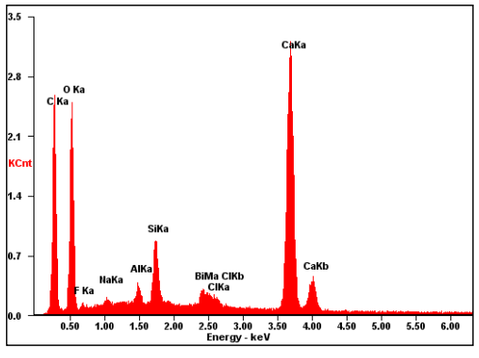


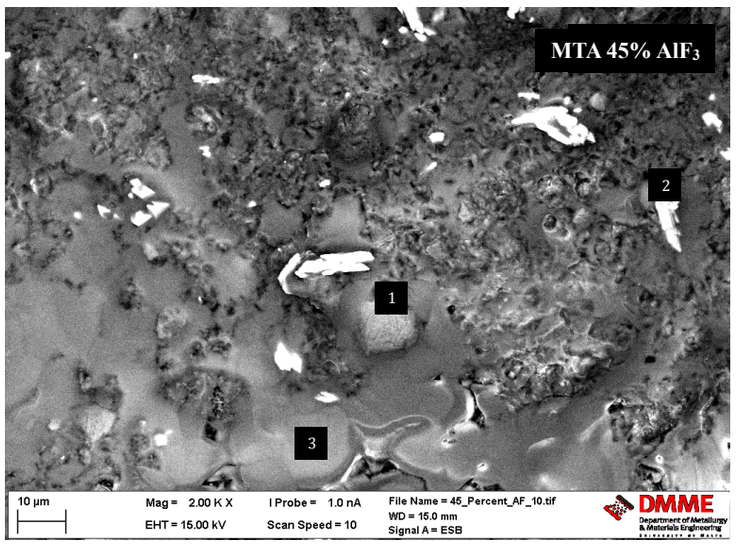












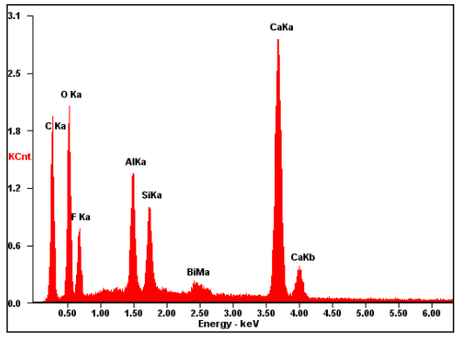
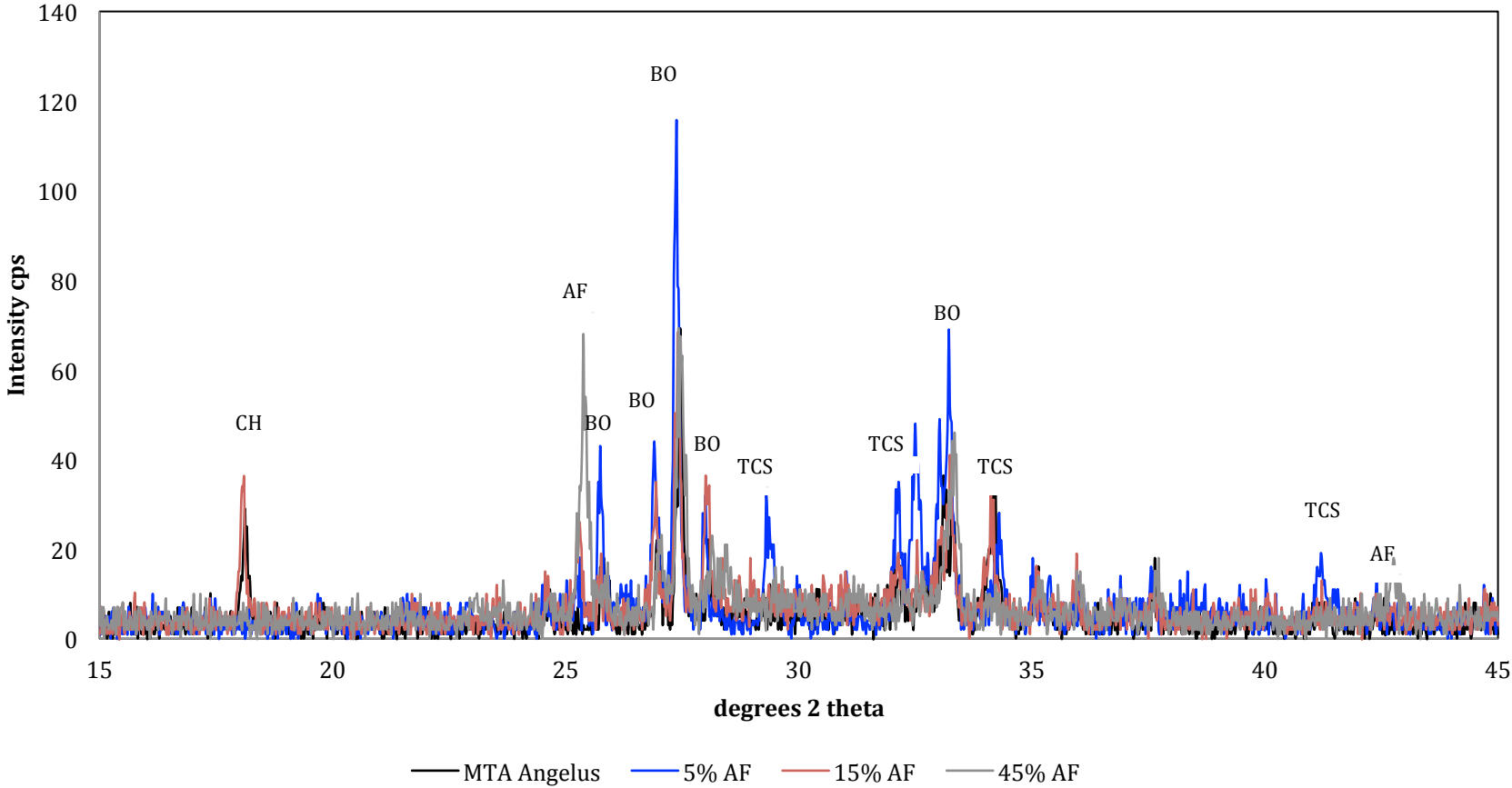
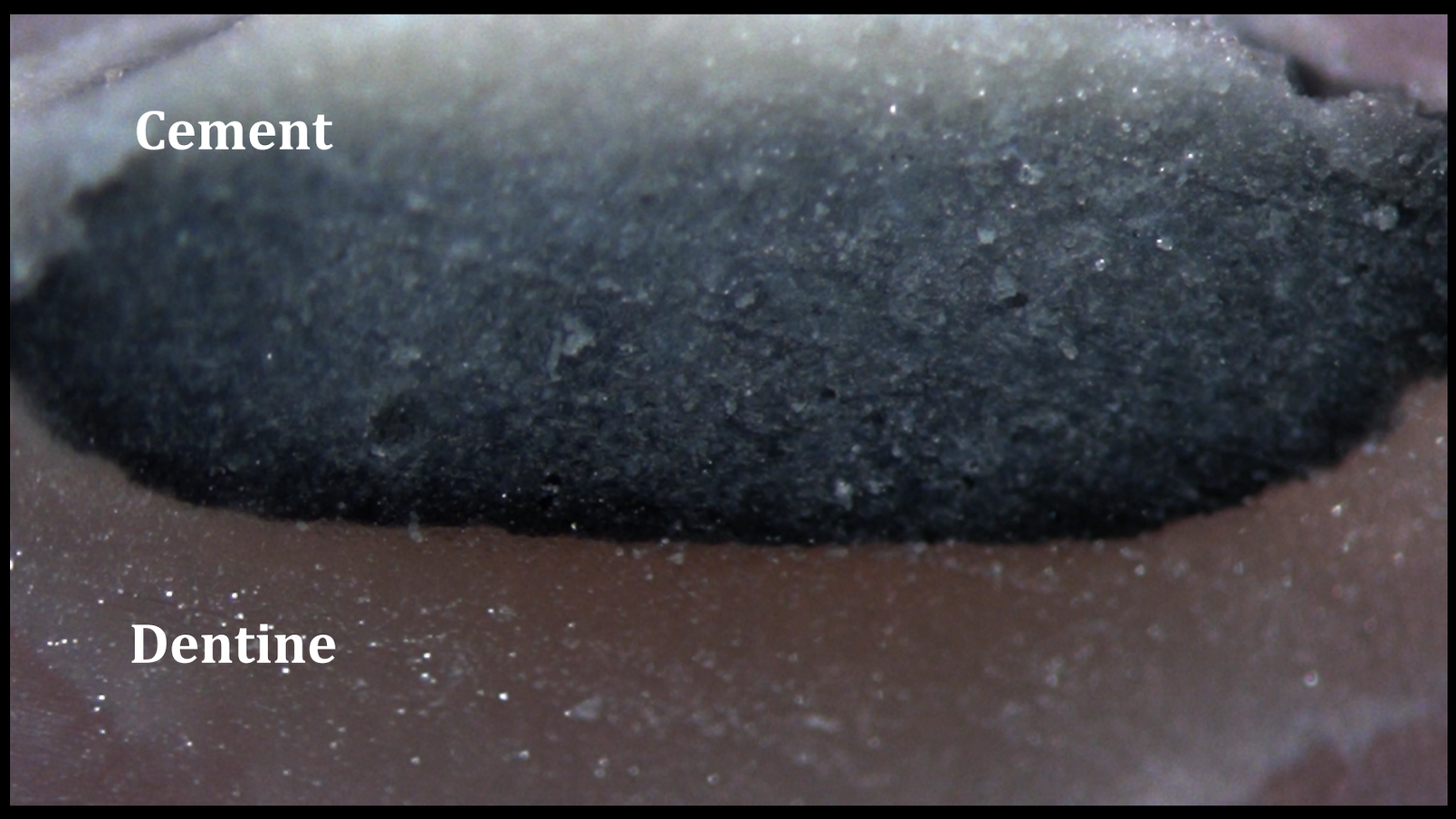


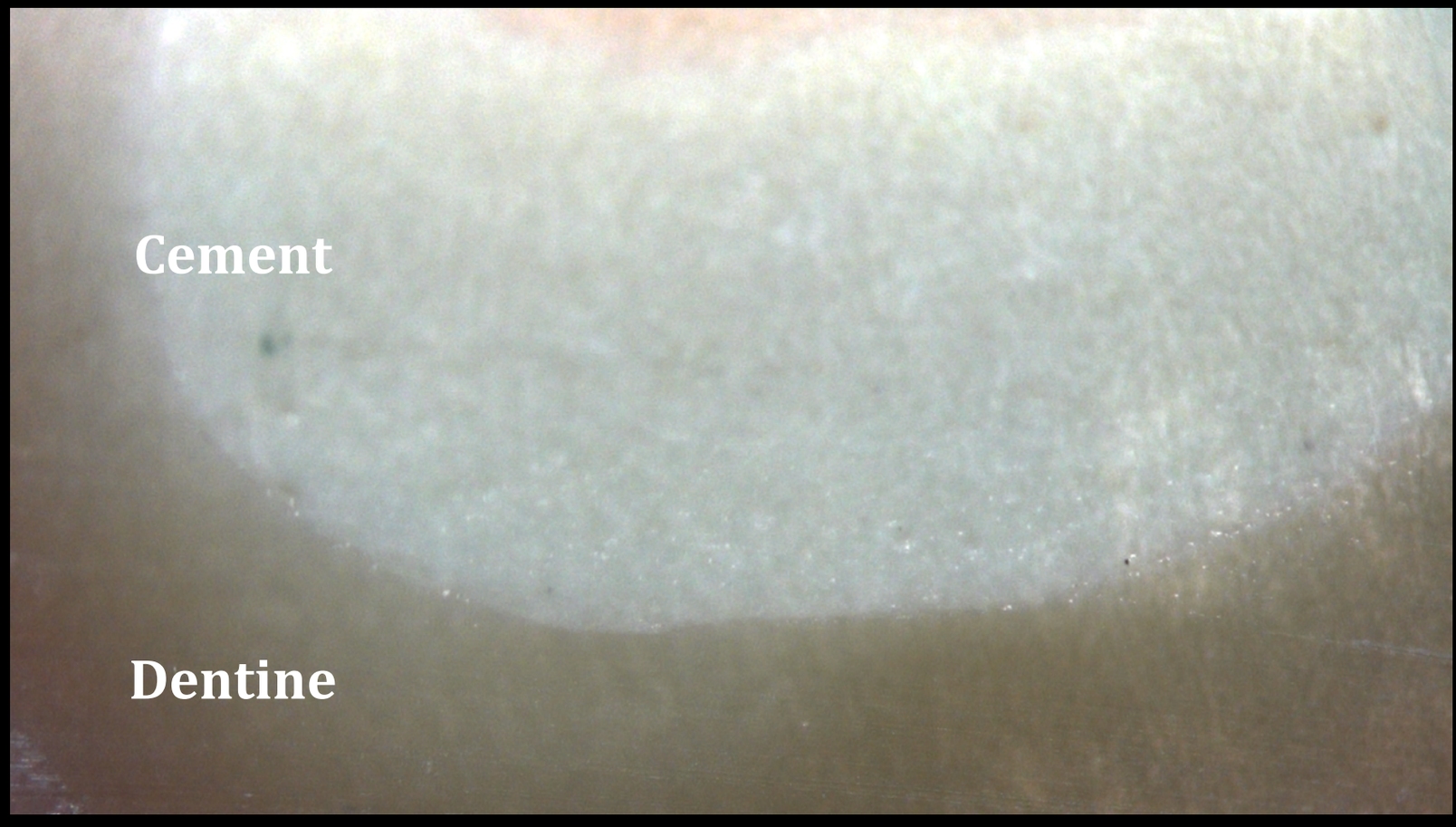
Figure 1 - Scanning electron micrographs and energy dispersive spectroscopic scans of MTA Angelus (1 - cement matrix and 2 - bismuth oxide particle) and modified MTA Angelus with additions of aluminum fluoride (AlF_3) in increasing proportions.


Intensity cps
degrees 2 theta
CH
AF
BO
BO
BO
BO
TCS
TCS
BO
TCS
TCS
AF
MTA Angelus
5% AF
15% AF
45% AF

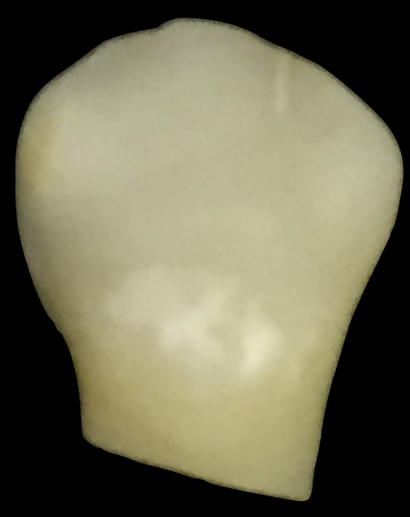


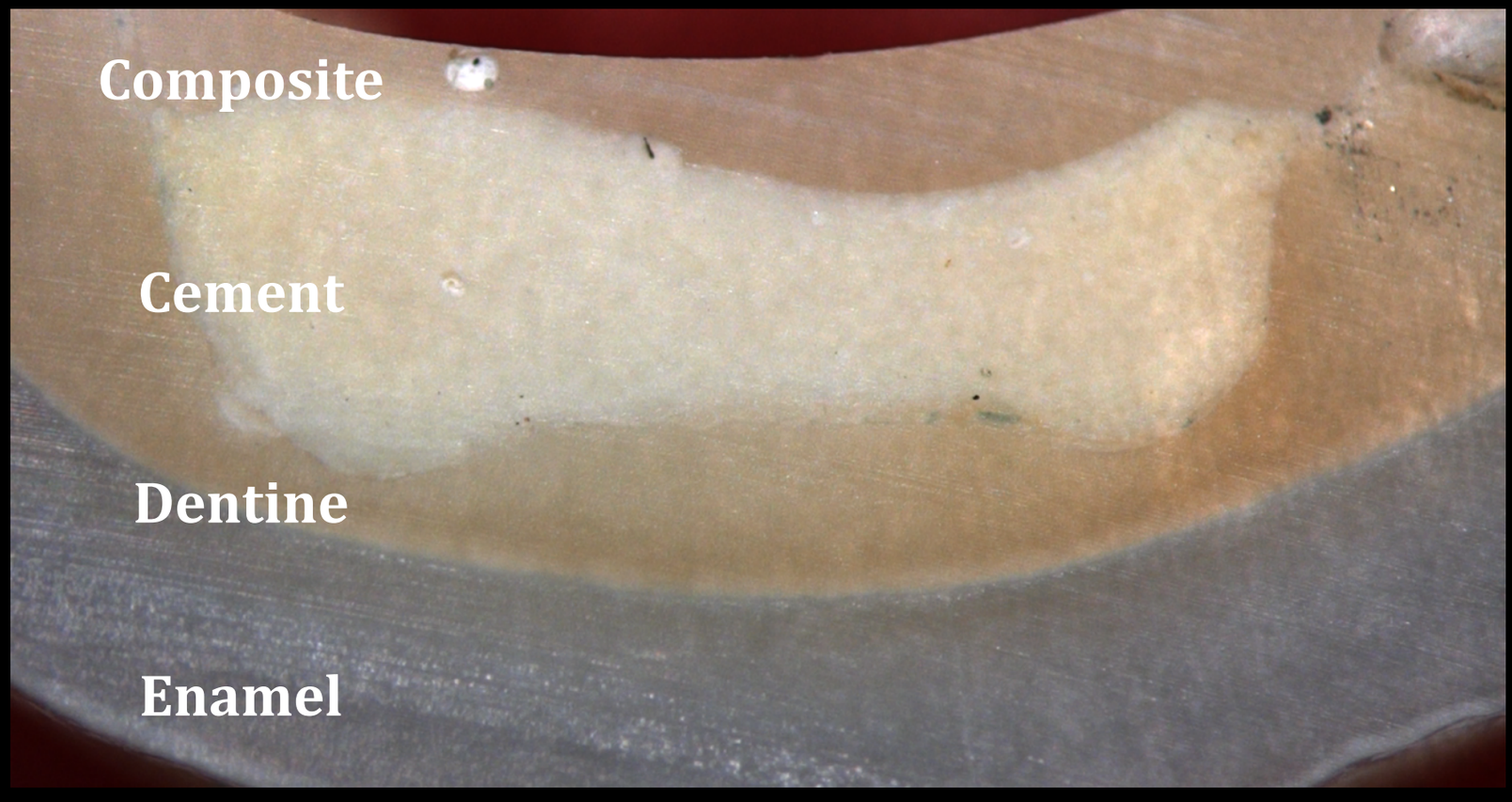
MTA Angelus
Human tooth

MTA Angelus
Bovine tooth



5% AlF_3
Human tooth





5% AlF_3
Bovine tooth

Figure 3 - Representative samples of human and bovine teeth filled using MTA Angelus and MTA containing 5% aluminum fluoride (5% AlF_3). The staining is evident in MTA Angelus group with color alteration of the material and dentine. The groups in which aluminum fluoride was added did not presented color alteration. Stereomicroscope images at x2 magnification.

Figure 4

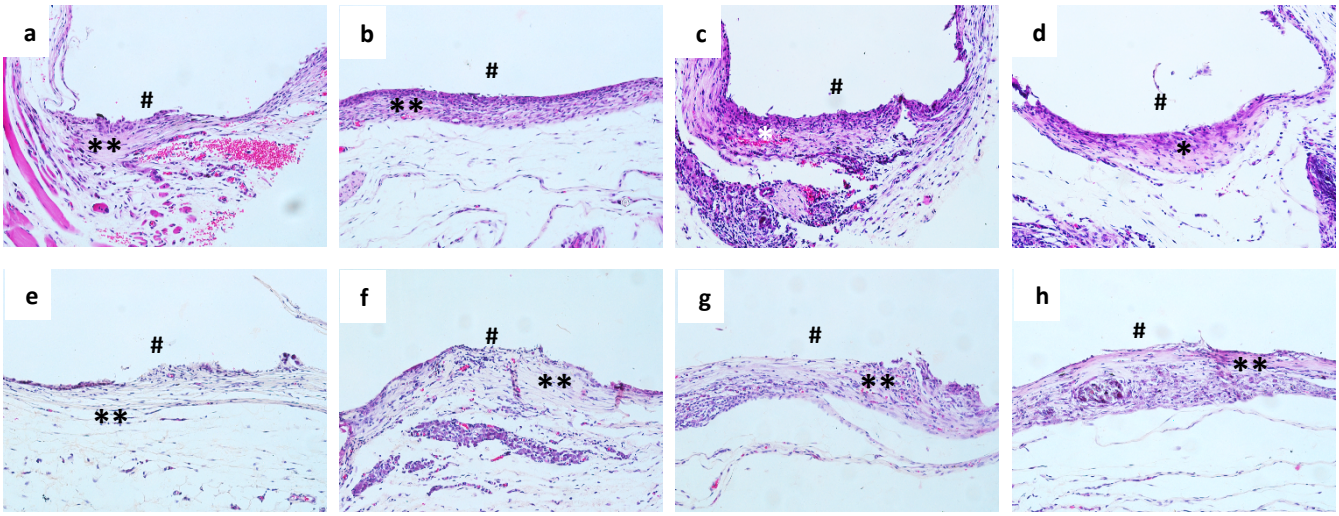


Figure 4 - (a-d) Microscopic representative specimens at 30 days of analysis. (a) MTA Angelus, (b) 5% AlF_3, (c) 15% AlF_3, (d) 45% AlF_3. Close to the material (#), inflammatory tissue (*) is present infiltrated by leucocytes. Areas with fibrous connective tissue in a capsule aspect (**) with slight infiltrate are observed (e-h) Microscopic representative specimens at 60 days of analysis. (e) MTA Angelus, (f) 5% AlF_3, (g) 15% AlF_3, (h) 45% AlF_3. At 60 days, is observed close to the material (#), inflammatory tissue (*) infiltrated by leucocytes. In some groups there was formation of fibrous capsule (**) with the presence of leucocytes. (HE; original magnification 20 x).

Table R1

Table 1 – Mean and standard deviation of physical tests (radiopacity, initial and final setting time and volume change) and color analysis (color change of human and bovine teeth and the lightness). Different lowercase letters in each column indicate statistically significant differences ($p < 0.05$).

Group	Radiopacity (mm Al)	Initial setting time (min)	Final setting time (min)	Volume change (%)	Color change (ΔE) human teeth	Color change (ΔE) bovine teeth	Lightness (L at 90d) human teeth	Lightness (L at 90d) bovine teeth
MTA Angelus	$4.4^{a} \pm 0.7$	$31^{a} \pm 4$	$67^{a} \pm 1$	$1.18^{ab} \pm 0.60$	$134.6^{a} \pm 42.8$	$106.8^{a} \pm 109.2$	$77.0^{a} \pm 5.7$	$80.6^{a} \pm 4.4$
5% AlF_3	$4.1^{a} \pm 1.3$	$14^{b} \pm 4$	$66^{a} \pm 14$	$1.07^{ab} \pm 0.88$	$13.4^{b} \pm 20.8$	$37.7^{b} \pm 20.4$	$82.2^{ab} \pm 7.0$	$91.2^{ab} \pm 1.9$
15% AlF_3	$3.6^{a} \pm 1.3$	$16^{b} \pm 3$	$95^{b} \pm 12$	$0.76^{a} \pm 0.53$	$50.6^{b} \pm 53.6$	$31.6^{b} \pm 4.9$	$87.5^{ab} \pm 2.2$	$92.5^{b} \pm 2.4$
45% AlF_3	$3.1^{a} \pm 1.6$	$33^{a} \pm 3$	$107^{b} \pm 3$	$2.24^{b} \pm 1.54$	$14.4^{b} \pm 3.1$	$45.8^{b} \pm 5.3$	$87.9^{b} \pm 3.2$	$93.5^{b} \pm 2.1$
Positive control	-	-	-	-	$1494.0^{c} \pm 176.4$	$1520.0^{c} \pm 28.4$	$44.5^{c} \pm 2.3$	$24.7^{c} \pm 19.0$
Negative control	-	-	-	-	$5.4^{d} \pm 1.0$	$6.3^{d} \pm 0.8$	$90.0^{b} \pm 1.7$	$90.7^{ab} \pm 1.3$

Table 2 - Mean and standard deviation of pH (ppm) and calcium ion release (mg L^{-1}) values in the periods of analysis. Different lowercase letters in each column indicate statistical differences among groups ($p < 0.05$).

Group	pH (ppm)			Calcium ion release (mg L^{-1})		
	3 h	24 h	28 d	3 h	24 h	28 d
MTA Angelus	$8.42^{a} \pm 0.26$	$8.02^{a} \pm 0.31$	$8.73^{a} \pm 0.45$	$3.08^{a} \pm 1.83$	$5.26^{a} \pm 1.35$	$8.90^{a} \pm 1.26$
5% AlF_3	$7.87^{b} \pm 0.17$	$8.58^{b} \pm 0.42$	$9.80^{b} \pm 0.79$	$5.31^{b} \pm 1.44$	$10.36^{b} \pm 1.18$	$21.65^{b} \pm 9.14$
15% AlF_3	$8.07^{b} \pm 0.13$	$8.93^{b} \pm 0.60$	$9.70^{b} \pm 0.97$	$4.88^{b} \pm 1.24$	$11.76^{b} \pm 2.69$	$17.28^{b} \pm 2.61$
45% AlF_3	$7.18^{c} \pm 0.02$	$7.15^{c} \pm 0.05$	$7.49^{c} \pm 0.19$	$2.63^{a} \pm 0.42$	$4.92^{a} \pm 1.39$	$19.28^{b} \pm 2.53$

Table 3 – Median and range of inflammatory infiltrate at 30 and 60 days of analysis. Different lowercase letters in each column indicate statistical differences among groups ($p < 0.05$).

Group	30 d	60 d
MTA Angelus	2.0^{a} (1.0-2.0)	2.0^{a} (1.0-3.0)
5% AlF_3	2.0^{a} (2.0-3.0)	3.0^{a} (1.0-3.0)

15% AlF_3	2.5[a] (2.0-3.0)	3.0[a] (2.0-3.0)
45% AlF_3	2.5[a] (2.0-3.0)	2.5[a] (2.0-3.0)