# Physicochemical, antimicrobial, and biological properties of White-MTAFlow

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Clinical Oral Investigations

# Physicochemical, antimicrobial and biological properties of White-MTAFlow

--Manuscript Draft--

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**Abstract:**

Objectives

To evaluate a new material containing tantalum oxide as an alternative radiopacifier, and a water-based gel for hydration.

Materials and Methods

ProRoot MTA (Dentsply), Biodentine (Septodont), and a new hydraulic calcium silicate cement White-MTAFlow (Ultradent) (in 'thin' consistency) were characterized and the interaction with dentin assessed. Physical and chemical properties radiopacity, setting time, linear flow, volumetric central filling, and lateral flow, pH, and volume change were investigated together with the color luminosity (L) and color change (ΔE). The agar diffusion and direct contact antimicrobial activity, and methylthiazolyldiphenyl-tetrazolium-bromide (MTT) cytotoxicity using human fibroblast cells were also evaluated. Data were statistically analyzed at a 5% significance level.

Results

All materials were composed of tricalcium and dicalcium silicate but had different radiopacifiers, and calcium hydroxide (portlandite) deposition was detected. White-

| | |
|---|---|
| | MTAFlow exhibited radiopacity values in accordance with ISO standard, and the longest setting time. The water-based gel provided the highest linear flow, a comparable cavity central filling, and the highest groove-lateral flow in the volumetric flow analysis. Alkalinity was observed in all materials, and similar volume loss for White-MTAFlow was assessed in comparison to Biodentine after 28-day immersion. White-MTAFlow presented the highest L value (91.5), and ProRoot MTA the lowest (78.1) due to dentin staining caused by bismuth migration. None of the materials presented inhibition halos against the tested bacteria, and similar turbidity values were obtained after 48 hours in direct contact with  E. faecalis,  indicating an up-regulation to bacterial growth. White-MTAFlow presented MTT cytocompatibility similarly to the control group.<br><br>Conclusions<br><br>White-MTAFlow in 'thin' consistency presents comparable physicochemical, biological, and antimicrobial properties to ProRoot MTA and Biodentine, and does not cause color alteration in dentin.<br><br>Clinical Relevance<br><br>White-MTAFlow is a suitable material for use as reparative endodontic cement. Further studies considering its biocompatibility are necessary. |
| **Response to Reviewers:** | Please find the attached response letter |
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Jul 15, 2020

Dear **Associated-Editor** and **Reviewers**,

We would like to kindly thank all the comments and the review of our manuscript. All of them helped us considerably to improve the manuscript. Please find all the alterations and suggestions applied to the reviewed version submitted after this first-review process.

In order to identify and efficiently track the alterations, I used the following colors in the 'revised manuscript' file:

- **Reviewer 1 comments** are highlighted in **red**;
- **Reviewer 2 comments** are highlighted in **blue**;

If, in your understanding, any other changes are necessary, please do not hesitate to contact us.

## Reviewer 1 comments' responses

- This study evaluated some of the physico-chemical, biological, and antimicrobial properties of White MTA Flow in thin constancy. This cement is a new material without much published work on its characterisation, which gives this paper the strength of novelty. However, I found it poorly written, with many details missing, especially in the materials & methods and results section. I would recommend rewriting some of the sections with consideration of the following:

Abstract:

Objectives: Not clear, should add "Evaluate a new…….. gel for hydration, in comparison with two calcium silicate-based cements; ProRoot MTA and Biodentine.

Materials and Methods: Did not mention what type of chemical characterisation was done

Results: Some details are added unnecessarily while the actual results are not mentioned: "Alkalinity was observed in all materials…" but findings were not listed

We appreciate the comment about the novelty of our paper. According to the reviewer suggestions regarding the abstract, the text was altered to:

**Section altered** *(abstract)*

**Objectives** To evaluate a new material containing tantalum oxide as an alternative radiopacifier, and a water-based gel for hydration, in comparison with two calcium silicate-based cement; ProRoot MTA and Biodentine.

**Materials and Methods** ProRoot MTA (Dentsply), Biodentine (Septodont), and a new hydraulic calcium silicate cement White-MTAFlow (Ultradent) (in 'thin' consistency) were characterized using x-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS). The interaction with dentin was also assessed using SEM and EDS.

**Results …** White-MTAFlow exhibited an alkalinity reduction and Biodentine, a progressive increase of pH values after 28 days. However, similar volume loss for White-MTAFlow was assessed in comparison to Biodentine after the 28-day immersion.

- Introduction: 1st paragraph: - "These materials should ideally…….biocompatibility and antimicrobial properties [1-4]" This is a very long sentence, should be divided into 2 or more sentences.

In consideration to the reviewer comment, the text was altered to:

**Section altered** *(introduction)*

These materials should ideally present physical characteristics such as color stability, radiopacity for follow-up exams, short setting time, low solubility, and flow during its working-time for material placement. Furthermore, chemical characteristics of alkalinity, release of calcium ions, and calcium hydroxide deposition after hydration is desirable to provide the biological properties of cell adhesion, biocompatibility, and antimicrobial properties [1–4].

- 4th paragraph: - "Biodentine (Septodont, Saint-Maur-des-Fossés, Île-de-France, France) initially developed for pulp capping, is composed of tricalcium silicate, dicalcium silicate…….polycarboxylate [8]." The reference cited here (Camilleri et al 2013) reported tricalcium silicate only, without the dicalcium silicate. The reference is incorrectly cited.

In observance to the reviewer comment regarding the composition, the reference was kept and the text corrected removing the 'dicalcium silicate' from the text:

**Section altered** *(introduction)*

Biodentine (Septodont, Saint-Maur-des-Fossés, Île-de-France, France) initially developed for pulp capping, is composed of tricalcium silicate, calcium carbonate, iron oxide, zirconium oxide (radiopacifier), and is mechanically mixed with water, calcium chloride, and polycarboxylate [5].

- Materials and Methods:

  1st paragraph:

  - "White-MTAFlow (Batch#20190220RD-01 [powder] and #2019022601 [liquid]) was prepared using 'thin' consistency ..": Write it as "was prepared in thin consistency".

Considering the reviewer comment, the text was altered to:

**Section altered** *(introduction)*

…White-MTAFlow (Batch#20190220RD-01 [powder] and #2019022601 [liquid]) was prepared in thin consistency (1 big-end plus 1 small-end spoon [0.19 g] to 3 drops).

- Why was not in thick consistency? As it will be comparable to the Biodentine consistency!

We very much appreciate this comment. In this study, we preferred to prepare the White-MTAFlow in thin consistency to get the highest possible flow properties from this new material, as the manufacturer stated that it is possible to apply the material using a syringe with quite small tips. Indeed, future studies should compare the White-MTAFlow properties prepared in all three consistencies (thin, thick, and putty). Another reason for the thin consistency used in the present study was to challenge the material solubility in immersion in its highest liquid to powder ratio, which potentially would exhibit the materials' highest possible solubility after 28 days.

**Section altered** *(none)*

- 2nd paragraph (Characterization of materials): - "Chemical characterized was performed…". Should be "Chemical characterization was performed…"
- No details about the SEM and EDS devices used (details are mentioned in the following paragraph, while they should be here).

In consideration to the reviewer comments, the text was altered to:

**Section altered** *(Materials and methods)*

**Characterization of materials**

Chemical characterization was performed on raw powder and crushed hydrated samples after 28-day immersion in Hank's Balanced Salt Solution (HBSS) using x-ray diffraction (XRD) Bruker D8 Advance (BrukerCorp, Billerica, MA, USA) scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS) (JSM-5600/LvJEOL, Tokyo, Japan) according to a previously methodology [6].

- 3rd paragraph (Evaluation of cement to dentine interface and elemental migration): - What was the storage medium of the samples used for dentine-cement characterisation?

Important missing information was included in this point, and the text was altered to:

**Section altered** *(Materials and methods)*

**Evaluation of cement to dentine interface and elemental migration**

The materials were placed over bovine enamel-dentine blocks in contact with dentin, and the blocks were acid-conditioned in the edges, adhesive coated and sealed with the composite resin before water-immersion. After 90-days, one sample of each group was transversally cut, embedded, and polished for SEM and EDS for elemental migration analysis in the cement/dentin interface [2].

- 4th paragraph (Radiopacity): - The size of the samples was mentioned twice (10x1) (Lines 47 and 50)

We apologize for this repetition; the text was altered to:

**Section altered** *(Materials and methods)*

Radiopacity was evaluated according to ISO-6876:2012 standard using 10x1 mm cement' disks (n = 3) and radiographed according to a previous methodology [7]. Metallic rings were used to shape the cement specimens.

- 5th paragraph (setting time) - No description about how the initial and final setting time was measured using the Gilmore needle.
  - No need to mention the ISO again, already mentioned before.

Further information about the setting time analysis was provided, and the text was altered to:

**Section altered** *(Materials and methods)*

**Setting time**

Setting initial and final times were evaluated using 10 x 2 mm cement' disks (n = 3) using Gilmore needles (ASTM-266/2008) in a temperature and humidity-controlled room at 37°C ± 1°C and 95% ± 5%, respectively [8]. Freshly mixed cement was placed into metallic rings were kept and periodically subjected to vertical pressure by using the Gilmore needles in which a 113.4 g needle was used for initial setting time and a 453.6 g for final setting time. The setting time (in minutes) was determined from the mixing to the moment that it was no longer possible to observe the marking of each needle on the surface of the specimens.

- 6th paragraph (flowability) - No details were given about the volumetric changes using the Micro CT, no details about the imaging machine and set up. They are essential to mention and explain how it was done.
  - No need to mention the ISO again, already mentioned before.

MicroCT information was included, and we kindly observe that the ISO information is important in this point for the reader to understand that two methods were used for flow analysis. The text was altered to:

**Section altered** *(Materials and methods)*

**Flowability**

The linear flow was evaluated using ISO-6876:2012 standard, and for volumetric assessment, a previously reported methodology [9]. A glass plate model (n = 3) was used with a central cavity and four lateral grooves. Using a graduated syringe, 0.05 ± 0.005 mL of each material was placed in the central

cavity, and a flat glass plate and weight (120 g in total) were placed over the material. Micro-computed tomography (µCT) was used for evaluation. The specimens were scanned using a µCT scanner (SkyScan 1174, Bruker, Kontich, Belgium) with a 0.5-mm aluminum filter, 31.03-µm pixel size, 360° rotation with 1.0-degree step, reconstructed with NRecon software (Bruker, Kontich, Belgium) and CTan software (Bruker, Kontich, Belgium) region of interest tool was used for analysis. The lateral volumetric flow was represented by the average material volume of the 2-mm far from the central cavity of the four lateral grooves (in $mm^3$). Central cavity filling was obtained considering the volume of material present in this region (in $mm^3$).

- 7th paragraph (pH and volumetric change in solution):
  - What is meant by "volumetric change in solution"?
  - It is mentioned that a simulated cavity was used for the retro-filling, how were the samples prepared here? No details!
  - How was the pH measured, and what was the storage conditions of the samples?

We appreciate this comment since important information and details were missing. The text was altered to:

**Section altered** *(Materials and methods)*

**pH and volumetric change**

The pH analysis and volumetric change after immersion were evaluated using a cement amount compatible with a simulated standardized root-end cavity (3x1 mm) (n = 3) in acrylic teeth after 3 hours, 24 hours and 28 days after immersion in distilled water. The cavities were filled with the tested cement and immersed in individual flasks containing 10 mL of deionized water. For analysis of pH, a calibrated pH-meter (371; Micronal, Sao Paulo, Brazil) was used, and measurements performed in each period. Before and after 28-day immersion, µCT scans (using the same previously described settings for flow) were used to establish the percentage of volumetric change [8].

- 8th paragraph (color assessment):

- Why the bonding agent was added? What was the purpose? In addition, it is not clear where it was applied. Is it meant on the cavo-surface angle or on the lateral walls of the cavity?
- Add mm after 5.0 (Line 35)

The bonding agent was added to ensure the sealing of the composite resin simulating a restorative procedure. If water infiltrated

and contacted the cement, the color analysis could be compromised once we aimed to solely evaluate the color parameters L and ΔE at this point. The enlightenments about the bonding agent placement were rewritten:

**Section altered** *(Materials and methods)*

**Color assessment**

Bovine enamel-dentin 3.5 mm-thick blocks of 10x10 mm (n = 5) were prepared with a 5.0 mm diameter, and 1.5-mm depth cavity in the dentinal surface and only its external cavity-edges were acid-etched, rinsed, and adhesive layer brushed. The bottom of the dentinal surface was not acid-etched, nor adhesive was used to provide a dentin/material direct contact.

- 9th paragraph (Antimicrobial Activity)

  - "Cement solubility was parallelly evaluated without bacteria and subtracted from expressed results". How the solubility was assessed? No details given!

In this regard, relevant information and details were missing. The text was altered to:

**Section altered** *(Materials and methods)*

**Antimicrobial activity**

Antimicrobial activity was evaluated considering agar diffusion inhibition halos in triplicates against *Enterococcus faecalis* (ATCC-29212), and *Porphyromonas gingivalis* (ATCC-49417) cultivated separately [10]. For direct contact test, 0,10 g of set material (n = 3) was evaluated using a calibrated spectroscope (Spectronic 20d+, Milton-Roy, Houston, USA) at 800 nm directly in contact with a planktonic *E. faecalis* culture and evaluated after 3, 24 and 48 hours [10]. The positive control was bacterial growth and negative control chlorhexidine 2%. Cement dissolution was considered parallelly using similar tubes, also containing 0.10 g cement samples in BHI, but without bacterial inoculum. The values of dissolution turbidity obtained from these parallel tubes were subtracted from the expressed results.

- Results: 1- Characterization of materials:

  The results are not sufficiently and properly presented:

  - No comparison for hydrated vs non-hydrated materials

  - EDS and XRD results not presented

In consideration to the reviewer comment, XRD details were described, and the text was altered to:

**Section altered** *(Results)*

**Characterization of materials**

The chemical characterization XRD plots, SEM/EDS images are shown in Fig. 1. XRD detected on the overlapped peaks of the raw powder and the crushed hydrated samples of Biodentine tricalcium silicate (ICCD: 00-031-0301), the radiopacifier zirconium oxide (ICCD: 00-037-1484), and after 28-day HBSS immersion calcium hydroxide (portlandite) (ICCD: 01-078-0315). ProRoot MTA exhibited tricalcium silicate (ICCD: 01-073-0599), the radiopacifier bismuth oxide (ICCD: 00-027-0053), and after 28-day HBSS immersion portlandite (ICCD: 01-070-5492). White-MTAFlow exhibited tricalcium silicate (ICCD: 00-013-0209), the radiopacifier tantalum oxide (ICCD: 00-005-0315), and after 28-day HBSS immersion portlandite (ICCD: 00-002-0969) deposition. SEM/EDS of hydrated cement revealed particles of cement interposed by particles of radiopacifier for all the tested materials.

- 2- Cement-Dentine interface:

  " For Biodentine, an overlap of ions zirconium and phosphorus…". This sentence is not clear? More findings need to be mentioned here regarding the migration and mapping of other ions.

Further details were described considering the ion migration for each material, and the text was altered to:

**Section altered** *(Results)*

**Evaluation of cement to dentine interface and elemental migration**

SEM and elemental mapping of the cement/dentin interface are shown in Fig. 2. ProRoot MTA exhibited the migration of bismuth to the adjacent dentin layer along with silicon ions and caused dentin darkening visible through enamel surface with tubular penetration. In White-MTAFlow, tantalum ions remained more in its cement matrix in comparison to its adjacent dentin layer. For Biodentine, as a limitation of the EDS, the overlap of ions zirconium and phosphorus peaks foreclosed the material/dentine interface analysis for this material.

- 3- Physical and chemical properties:

  - Better to remain consistent about the subheadings, so either all the properties tested are listed under the same subheading in the materials and methods, or the opposite.

The headings and subheadings were matched according to the MM section:

**Section altered** *(Results)*

**Radiopacity, setting time, flowability, pH and volumetric change**

- "Biodentine, although presented a gradual increase during analysis, its pH average was smaller than….": use "lower than" instead of "smaller than"

The text was altered to:

**Section altered** *(Results)*

Biodentine, although exhibited a gradual increase during analysis, its pH average was lower than those obtained by ProRoot MTA and White-MTAFlow.

## Reviewer 2 comments' responses

- This study investigated the physical, chemical, antibacterial, as well as cytotoxicity of a tantalum oxide containing hydraulic calcium silicate cement. The paper is in general well structured, I have some comments:
- 1 How innovative is the material tested as similar properties were observed as compared to ProRoot MTA and Biodentine, the setting time was longer than Biodentine and ProRoot MTA.

We kindly appreciate the comments about our paper. The purpose of the study was to verify the properties of this new material which is an evolution of Grey-MTAFlow (Ultradent). Indeed several properties of the White-MTAFlow were similar to the other tested materials, although setting time was probably higher in White-MTAFlow due to the 'thin' consistency tested in the present study as a result of the liquid to powder ratio used. The choice for this consistency in the present study aimed to obtain the highest possible flow for this material. Certainly, futures studies should compare the two other consistencies (thick and putty) recommended by the manufacturer, and this comparison would be possible

regarding the setting time. The possibility to use a powder/liquid formulation in a syringe with different delivery-points is innovative and should be considered for clinical use once the material hydration is tightly controlled by the professional during the material preparation.

**Section altered** *(discussion)*

Short initial setting time in balance with proper working time for cement insertion is essential [11]. Biodentine and ProRoot MTA exhibited a shorter initial setting time in comparison to White-MTAFlow in 'thin' consistency. In future studies, the use of 'thick' or 'putty' consistencies would probably present different setting times for White-MTAFlow, once the powder to liquid ratio would be different from the one used in the present study.

- 2 The description for the materials and results should be more in details. It is in general for all the materials and methods.

  Taking 'evaluation of cement to dentine interface and elemental migration' as an example, why was the specimen stored for 90 days, what was the storage condition?

We kindly observe that both sections, 'Material and methods' and 'Results,' were rewritten in a more detailed manner since important information was missing, and these alterations are highlighted in red as these alterations were also observed by the Reviewer #1.

The storage for 90 days considered a previously reported method [2] simulating a long-term dentin/material contact in which both color alteration and ion migration could be assessed.

**Sections altered** *(Materials and methods* and *Results)*

**Evaluation of cement to dentine interface and elemental migration**

The materials were placed over bovine enamel-dentine blocks in contact with dentin, and the blocks were acid-conditioned in the edges, adhesive coated and sealed with the composite resin before water-immersion. After 90-days, one sample of each group was transversally cut, embedded, and polished for SEM and EDS for elemental migration analysis in the cement/dentin interface [2].

- In the result, it was mentioned that "ProRoot MTA caused dentin darkening visible through enamel surface and with tubular penetration", which figure

precisely indicated tubular penetration and dentin darkening was not mentioned. Only one specimen was tested for each group, is it enough?

In consideration of the reviewer comment, figure 2d was indicated for ProRoot MTA. The analysis of dentin/material interface was performed in selected sectioned samples for each material and is complemented with the XRD analysis in the present study. Since the results were in agreement (considering components and ions), one sample was representative of the dentin/material interface analysis. The text was altered to:

**Section altered** *(Results)*

**Evaluation of cement to dentine interface and elemental migration**

SEM and elemental mapping of the cement/dentin interface are shown in Fig. 2. ProRoot MTA exhibited the migration of bismuth to the adjacent dentin layer along with silicon ions and caused dentin darkening (Fig. 2d) visible through enamel surface with tubular penetration.

- 3 The cytotoxicity assay was also not described in details?
  Were the cements completely set before exposing the cells?

Further details were included, according to the reviewer's comment. The text was altered to:

**Section altered** *(Materials and methods)*

**Cytotoxicity**

Cytotoxicity was evaluated using methylthiazolyldiphenyl-tetrazolium bromide (MTT) on freshly-extracted human third-molar periodontal ligament fibroblasts (B041-Periocells, FOP-Unicamp, Periodontics Division, SP, Brazil [Research Ethics Committee code: CAAE 20189119.7.0000.5418]). Immediately after mixing, materials were placed in a 3 x 1 mm root-end sterile experimental cavity (n = 3) directly in contact with the culture cell (5.300 cells/cm$^2$) in Dulbecco's modified Eagle's medium (DMEM) and antibiotics [12]. Absorbance was measured at 490 nm, and the cell viability percentage for each test material was calculated after 24 hours in comparison to the negative control group. Three independent experiments were carried out to ensure the reproducibility of the results.

**References (cited in this response letter)**


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**Physicochemical, antimicrobial and biological properties of White-MTAFlow**

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# Physicochemical, antimicrobial and biological properties of White-MTAFlow


## Abstract

**Objectives** To evaluate a new material containing tantalum oxide as an alternative radiopacifier, and a water-based gel for hydration, in comparison with two calcium silicate-based cement; ProRoot MTA and Biodentine.

**Materials and Methods** ProRoot MTA (Dentsply), Biodentine (Septodont), and a new hydraulic calcium silicate cement White-MTAFlow (Ultradent) (in 'thin' consistency) were characterized using x-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS). The interaction with dentin was also assessed using SEM and EDS. Physical and chemical properties radiopacity, setting time, linear flow, volumetric central filling, and lateral flow, pH, and volume change were investigated together with the color luminosity (L) and color change (ΔE). The agar diffusion and direct contact antimicrobial activity, and methylthiazolyldiphenyl-tetrazolium-bromide (MTT) cytotoxicity using human fibroblast cells were also evaluated. Data were statistically analyzed at a 5% significance level.

**Results** All materials were composed of tricalcium and dicalcium silicate but had different radiopacifiers, and calcium hydroxide (portlandite) deposition was detected in XRD analysis. White-MTAFlow exhibited radiopacity values in accordance with ISO standard, and the longest setting time. The water-based gel provided the highest linear flow, a comparable cavity central filling, and the highest groove-lateral flow in the volumetric flow analysis. White-MTAFlow exhibited an alkalinity reduction and Biodentine, a progressive increase of pH values after 28 days. However, similar volume loss for White-MTAFlow was assessed in comparison to Biodentine after the 28-day immersion. White-MTAFlow showed the highest L value (91.5), and ProRoot MTA the lowest (78.1) due to dentin staining caused by bismuth migration. None of the materials exhibited inhibition halos against the tested bacteria, and similar turbidity values were obtained after 48 hours in direct contact with *E. faecalis*, indicating an up-regulation to bacterial growth. White-MTAFlow showed MTT cytocompatibility similarly to the control group.

**Conclusions** White-MTAFlow in 'thin' consistency presents comparable physicochemical, biological, and antimicrobial properties to ProRoot MTA and Biodentine, and does not cause color alteration in dentin.

**Clinical Relevance** White-MTAFlow is a suitable material for use as reparative endodontic cement. Further studies considering its biocompatibility are necessary.


**Key Words** White-MTAFlow · ProRoot MTA · Biodentine · Hydraulic calcium silicate cement

## Introduction

Hydraulic calcium silicate-based endodontic materials are used for vital pulp therapies, regenerative endodontic procedures, apical plugs, corrective procedures (i.e., perforations and root resorptions), and surgical root-end filling. These materials should ideally present physical characteristics such as color stability, radiopacity for follow-up exams, short setting time, low solubility, and flow during its working-time for material placement. Furthermore, chemical characteristics of alkalinity, release of calcium ions, and calcium hydroxide deposition after hydration is desirable to provide the biological properties of cell adhesion, biocompatibility, and antimicrobial properties [1–4]. The available materials contemplated several of these properties for a hydraulic calcium silicate cement, but others still need improvements such as its color stability [2], difficulty in handling, and flow [5].

These materials present a hydraulic nature and, thus, set and improve their properties in the presence of moisture [6]. Hydraulic calcium silicate cement is composed of tricalcium and dicalcium silicate, and a radiopacifier [6, 7]. Additives can be included in the powder, such as the calcium carbonate in Biodentine or its' liquid such as polycarboxylate [8], water-based gel enhancing the material handling [1, 5], and calcium chloride [8] reduces the cement setting time. This additive demands to test once they can alter the material properties.

Color stability after cement placement, especially in esthetic regions, is expected from these materials [2, 9]. ProRoot MTA (Dentsply, Tulsa, OK) was the first commercially available material and is composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate, bismuth oxide (radiopacifier), and is mixed with distilled water [10]. The presence of bismuth oxide in hydraulic calcium silicate compositions causes dentinal staining due to the reaction with collagen present in the organic dentin matrix [2], which is a shortcoming of this formulation.

Biodentine (Septodont, Saint-Maur-des-Fossés, Île-de-France, France) initially developed for pulp capping, is composed of tricalcium silicate, calcium carbonate, iron oxide, zirconium oxide (radiopacifier), and is mechanically mixed with water, calcium chloride, and polycarboxylate [8]. The liquid contains calcium chloride to reduce the setting time [11] and water-soluble polymers based on polycarboxylate in order to increase the plasticity of the material. The development of this cement sought to aggregate the biocompatibility of calcium silicate-based cement with a short setting time, and the use of an alternative inert radiopacifier (zirconium oxide) that does not stain dental structures [8, 12]. Previous clinical studies have shown that Biodentine presents results similar to ProRoot MTA [13–15].

White-MTAFlow (Ultradent Products Inc., South Jordan, UT) is a new material composed of tricalcium silicate, dicalcium silicate, tantalum oxide (radiopacifier) and mixed with a water-based gel containing silicone. The predecessor of this Ultradent material was grey and contained bismuth oxide as a radiopacifier [5]. In this new white material version, the raw powder color used for its manufacture was altered to white, and the former radiopacifier was replaced with tantalum oxide in order to avoid potential tooth discoloration [2]. The water-based gel used for material hydration was kept, along with the possibility of the material to be used in three consistencies: 'thin,' 'thick,' and 'putty.' The first two are usable in syringes, which is an innovative delivery method considering clinical use. No previous studies evaluated White-MTAFlow properties.

This study aimed to evaluate using the 'thin' consistency the physicochemical, biological, and antimicrobial properties of White-MTAFlow, and to compare these properties to those of ProRoot MTA and Biodentine. The hypotheses tested are, first, that White-MTAFlow exhibits similar properties in comparison to the other tested materials and, second, this cement does not cause dental discoloration once bismuth oxide was replaced with tantalum oxide in this new composition.

## Materials and methods

ProRoot MTA (Batch#180414) and Biodentine (Batch#B22869) were prepared according to manufacturers' instructions, and White-MTAFlow (Batch#20190220RD-01 [powder] and #2019022601 [liquid]) was prepared in thin consistency (1 big-end plus 1 small-end spoon [0.19 g] to 3 drops).

### Characterization of materials

Chemical characterization was performed on raw powder and crushed hydrated samples after 28-day immersion in Hank's Balanced Salt Solution (HBSS) using x-ray diffraction (XRD) Bruker D8 Advance (BrukerCorp, Billerica, MA, USA) scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS) (JSM-5600/LvJEOL, Tokyo, Japan) according to a previously methodology [16]. Phase identification was accomplished using search-match software from the International Centre for Diffraction Data (ICDD, Newtown Square, PA, USA).

### Evaluation of cement to dentine interface and elemental migration

The materials were placed over bovine enamel-dentine blocks in contact with dentin, and the blocks were acid-conditioned in the edges, adhesive coated and sealed with the composite resin before water-immersion. After 90-days, one sample of each group was transversally cut, embedded, and polished for SEM and EDS for elemental migration analysis in the cement/dentin interface [2]. Representative samples of each material were also transversally sectioned and viewed under the stereomicroscope (SZX9, Olympus, Tokyo, Japan) at ×2 magnification. The images were acquired in software AxioVision (Carl Zeiss, Jena, Germany).

### Radiopacity

Radiopacity was evaluated according to ISO-6876:2012 standard using 10x1 mm cement' disks (n = 3) and radiographed according to a previous methodology [7]. Metallic rings were used to shape the cement specimens. Samples were kept at 37 ± 1°C and relative humidity until its final setting, removed from the rings and radiographed with a digital sensor (to avoid film processing effects) (Micro Imagem, Indaiatuba, Sao Paulo, Brazil) using the settings of 60 kV, 10 mA, 0.3-second exposure, and focus-film distance of 30 cm. An aluminum scale with progressive thickness was used comparatively. Values

obtained in the grey-scale were converted into aluminum equivalent thickness (mm Al) using the Image J software (National Institutes of Health, Bethesda, MD, USA).

**Setting time**

Setting initial and final times were evaluated using 10 x 2 mm cement' disks (n = 3) using Gilmore needles (ASTM-266/2008) in a temperature and humidity-controlled room at 37°C ± 1°C and 95% ± 5%, respectively [17]. Freshly mixed cement was placed into metallic rings were kept and periodically subjected to vertical pressure by using the Gilmore needles in which a 113.4 g needle was used for initial setting time and a 453.6 g for final setting time. The setting time (in minutes) was determined from the mixing to the moment that it was no longer possible to observe the marking of each needle on the surface of the specimens.

**Flowability**

The linear flow was evaluated using ISO-6876:2012 standard, and for volumetric assessment, a previously reported methodology [18]. A glass plate model (n = 3) was used with a central cavity and four lateral grooves. Using a graduated syringe, 0.05 ± 0.005 mL of each material was placed in the central cavity, and a flat glass plate and weight (120 g in total) were placed over the material. Micro-computed tomography (µCT) was used for evaluation. The specimens were scanned using a µCT scanner (SkyScan 1174, Bruker, Kontich, Belgium) with a 0.5-mm aluminum filter, 31.03-µm pixel size, 360° rotation with 1.0-degree step, reconstructed with NRecon software (Bruker, Kontich, Belgium) and CTan software (Bruker, Kontich, Belgium) region of interest tool was used for analysis. The lateral volumetric flow was represented by the average material volume of the 2-mm far from the central cavity of the four lateral grooves (in $mm^3$). Central cavity filling was obtained considering the volume of material present in this region (in $mm^3$).

**pH and volumetric change**

The pH analysis and volumetric change after immersion were evaluated using a cement amount compatible with a simulated standardized root-end cavity (3x1 mm) (n = 3) in acrylic teeth after 3 hours, 24 hours and 28 days after immersion in distilled water. The cavities were filled with the tested cement and immersed in individual flasks containing 10 mL of deionized water. For analysis of pH, a calibrated pH-meter (371; Micronal, Sao Paulo, Brazil) was used, and measurements performed in each period. Before and after 28-day immersion, µCT scans (using the same previously described settings for flow) were used to establish the percentage of volumetric change [17].

**Color assessment**

Bovine enamel-dentin 3.5 mm-thick blocks of 10x10 mm (n = 5) were prepared with a 5.0 mm diameter, and 1.5-mm depth cavity in the dentinal surface and only its external cavity-edges were acid-etched, rinsed, and adhesive layer brushed. The bottom of the dentinal surface was not acid-etched, nor adhesive was used to provide a dentin/material direct contact. Cavities were then filled with freshly mixed cement and after its setting, sealed using light-curing composite resin. Triple antibiotic paste and unfilled samples served as controls. Samples storage occurred separately in the water at 37°C. Luminosity (L) and color change (ΔE) after 24 hours, 28 days, and 90 days were evaluated using a spectrophotometer (EasyShade-VITA, Zahnfabrik, Germany). A controlled lighting room was used for assessments.

**Antimicrobial activity**

Antimicrobial activity was evaluated considering agar diffusion inhibition halos in triplicates against *Enterococcus faecalis* (ATCC-29212), and *Porphyromonas gingivalis* (ATCC-49417) cultivated separately [19]. For direct contact test, 0,10 g of set material (n = 3) was evaluated using a calibrated spectroscope (Spectronic 20d+, Milton-Roy, Houston, USA) at 800 nm directly in contact with a planktonic *E. faecalis* culture and evaluated after 3, 24 and 48 hours [19]. The positive control was bacterial growth and negative control chlorhexidine 2%. Cement dissolution was considered parallelly using similar tubes, also containing 0.10 g cement samples in BHI, but without bacterial inoculum. The values of dissolution turbidity obtained from these parallel tubes were subtracted from the expressed results.

**Cytotoxicity**

Cytotoxicity was evaluated using methylthiazolyldiphenyl-tetrazolium bromide (MTT) on freshly-extracted human third-molar periodontal ligament fibroblasts (B041-Periocells, FOP-Unicamp, Periodontics Division, SP, Brazil [Research Ethics Committee code: CAAE 20189119.7.0000.5418]). Immediately after mixing, materials were placed in a 3 x 1 mm root-end sterile experimental cavity (n = 3) directly in contact with the culture cell (5.300 cells/cm$^2$) in Dulbecco's modified Eagle's medium (DMEM) and antibiotics [20]. Absorbance was measured at 490 nm, and the cell viability percentage for each test material was calculated after 24 hours in comparison to the negative control group. Three independent experiments were carried out to ensure the reproducibility of the results.

**Statistical analysis**

BioEstat 5.3 (Mamiraua-Tefe, Brazil) and SPSS Statistics 24 (IBM, Armonk, NY, USA) software were used. Data normality was assessed using Shapiro-Wilk at a significance level of 0.05. The sample size was determined for each test to express a test power of at least 80%. Unpaired T-test was used for parametric values (initial setting time, flow, pH, and volume change), whereas Mann-Whitney and Kolmogorov-Smirnov tests were used for non-parametric values (L, ΔE, radiopacity, final setting time, direct antimicrobial test, and cytotoxicity).

## Results

### Characterization of materials

The chemical characterization XRD plots, SEM/EDS images are shown in Fig. 1. XRD detected on the overlapped peaks of the raw powder and the crushed hydrated samples of Biodentine tricalcium silicate (ICCD: 00-031-0301), the radiopacifier zirconium oxide (ICCD: 00-037-1484), and after 28-day HBSS immersion calcium hydroxide (portlandite) (ICCD: 01-078-0315). ProRoot MTA exhibited tricalcium silicate (ICCD: 01-073-0599), the radiopacifier bismuth oxide (ICCD: 00-027-0053), and after 28-day HBSS immersion portlandite (ICCD: 01-070-5492). White-MTAFlow exhibited tricalcium silicate (ICCD: 00-013-0209), the radiopacifier tantalum oxide (ICCD: 00-005-0315), and after 28-day HBSS immersion portlandite (ICCD: 00-002-0969) deposition. SEM/EDS of hydrated cement revealed particles of cement interposed by particles of radiopacifier for all the tested materials.

### Evaluation of cement to dentine interface and elemental migration

SEM and elemental mapping of the cement/dentin interface are shown in Fig. 2. ProRoot MTA exhibited the migration of bismuth to the adjacent dentin layer along with silicon ions and caused dentin darkening (Fig. 2d) visible through enamel surface with tubular penetration. In White-MTAFlow, tantalum ions remained more in its cement matrix in comparison to its adjacent dentin layer. For Biodentine, as a limitation of the EDS, the overlap of ions zirconium and phosphorus peaks foreclosed the material/dentine interface analysis for this material.

### Radiopacity, setting time, flowability, pH and volumetric change

Radiopacity, setting time, and flow data are shown in Table 1. All tested cement achieved at least 5 mm Al surpassing the minimum required by the ISO standard. White-MTAFlow showed similar radiopacity results ($p = 0.121$) to ProRoot MTA.

White-MTAFlow had the highest initial and final setting time ($p < 0.001$) and the highest linear flow in both tested methods. Biodentine exhibited the highest volumetric cavity filling but without a significant difference to both ProRoot MTA ($p = 0.280$) or White-MTAFlow ($p = 0.306$).

The pH and volumetric changes are shown in Table 2. White-MTAFlow showed a significant alkalinity reduction after 28 days ($p = 0.002$) in comparison to 3-hour measurement. Biodentine, although it exhibited a gradual increase during analysis, its pH average was lower than those obtained by ProRoot MTA and White-MTAFlow. ProRoot MTA showed a slight increase in volume. White-MTAFlow and Biodentine had similar volumetric loss after 28 days ($p = 0.172$).

### Color assessment

Color alterations L and ΔE are expressed in Table 3. ProRoot MTA gradually reduced its L, and at 90 days obtained the lowest value (78.1) in comparison to Biodentine (82.2) ($p = 0.034$) and White-MTAFlow (91.5) ($p < 0.001$). ΔE showed that all tested materials exhibited color change after 90 days.

**Antimicrobial and cytotoxicity**

No inhibition halos were observed for the cement, regardless of the bacteria tested in the agar diffusion test. Biodentine exhibited a broader halo of cement solubility in comparison to the other materials and control. Turbidity is represented in Fig. 3. After 24 hours, all cement showed levels of turbidity higher than the graphic tendency bar after discounting their solubility when compared to both controls ($p < 0.05$).

Cytotoxicity, after 24 hours, is represented in Fig. 3. Biodentine exhibited the highest cytocompatibility values ($p < 0.001$). ProRoot MTA and White-MTAFlow had similar values ($p = 0.286$).

## Discussion

White-MTAFlow tested in the present study, is a new alternative to Grey-MTAFlow in which not only the raw powder was altered to white, but the bismuth oxide used as radiopacifier was replaced by tantalum oxide in this white version of the material. These formulations can be used in three consistencies: 'thin,' 'thick,' and 'putty.' The first two consistencies are usable in syringes, which is a facilitator delivery method considering its clinical use. White-MTAFlow is a hydraulic calcium silicate-based cement, and its hydration is accomplished with a water-based gel that provides in 'thin' consistency the highest flow of the material, which justified our choice of this powder to liquid ratio. Another reason for the use of this consistency was to challenge the material solubility in immersion in its highest liquid to powder ratio, which potentially would exhibit the materials' highest possible solubility after 28 days. In the present study, the properties of White-MTAFlow in 'thin' consistency are compared to the first available commercial formula, the ProRoot MTA, and Biodentine, which is a well-researched newer generation material.

The testing outline for the materials followed a previously reported sequence [21], which includes physicochemical tests, two basic antimicrobial tests (agar diffusion and direct contact), and the biological test (MTT) using human fibroblast cells. Volume change method after immersion [17] using μCT and simulated root-end cavities were preferred, once it is considered an amount of material compatible to clinical reality, along with the precision of μCT analysis, once these materials should present long-term stability to ensure the sealing necessary avoiding bacterial penetration. The fact that dental materials are continuously challenged microbiologically justify the inclusion of antimicrobial tests using different bacterial strains (*E. faecalis* and *P. gingivalis*) commonly encountered in endodontic infections [19]. There is not a specific ISO standard for these materials category, a fact which demands the use of previously reported methods, and ISO 6876:2012 (initially indicated for endodontic sealers) to evaluate new materials such as White-MTAFlow. These properties should be previously tested before

animal testing considering the principles of the 3Rs (Replacement, Reduction, and Refinement) in research [22].

Calcium hydroxide deposition after cement hydration is crucial to initiate the ensuing biological reactions of hydraulic calcium silicate-based materials [6]. Characterization in the present study used XRD, SEM, and EDS. Compound composition identification is possible using XRD analysis, and SEM/EDS only provides information about the elements present in the material without compound identification [23]. In the present study, all tested materials showed peaks of tricalcium silicate ($C_3S$) and the radiopacifier claimed by the manufacturer. Calcium hydroxide was detected in all samples after 28-day immersion in HBSS. Previous studies showed calcium hydroxide deposition for ProRoot MTA and Biodentine [24] corroborating with our findings.

Color stability and long-term esthetics outcomes are expected after clinical procedures. The color alteration of the tested materials was evaluated using a previous method [2]. ProRoot MTA had the lowest L values after 90 days. Studies demonstrated ProRoot MTA discoloration in contact with tooth and tissues (15-17), attributing this discoloration to bismuth oxide destabilization. Other bismuth-containing materials were also associated with discoloration [2, 25]. All samples showed ΔE variations, which corroborates with previous studies [2], indicating the importance of long-term tests considering this aspect. In the sectioned samples, there was a visible darkening for ProRoot MTA and EDS mapping evidenced bismuth migration. The Grey-MTAFlow that contains bismuth oxide as a radiopacifier potentially causes dentinal staining due to this radiopacifier, similar to the ProRoot MTA results obtained in the present study. The radiopacifier replacement with tantalum oxide in the White-MTAFlow is a significant modification considering the esthetic aspect once this material showed the highest L values after 90 days of evaluation.

Bismuth oxide has proper radiopacity characteristics due to its high molecular weight (465.96 g/mol), requiring small amounts to achieve an ideal radiopacity (7). Replacement of this compound could be a concern regarding radiopacity. White-MTAFlow containing tantalum oxide provided similar radiopacity results to ProRoot MTA. Biodentine exhibited the lowest radiopacity with similar results previously reported [1]. Grey-MTAFlow was previously reported with radiopacity around 5 mm Al [5], which corroborates with our findings for White-MTAFlow. The radiopacifier alteration in White-MTAFlow did not compromise its radiopacity.

ISO-6876:2012 is normative for root canal sealers evaluation, but hydraulic calcium silicate-based materials have different indications and consistencies. There is not a specific normative regarding these materials flow evaluation. However, a previously reported volumetric analysis [18] was used along with ISO linear testing in the present study to consider not only the lateral flow but also its central filling capacity. Higher flowability was expected for white-MTAFlow, due to the water-based gel in its liquid and the 'thin' consistency used. However, although this material showed ISO flow significantly higher than the other materials, a similar volumetric flow was detected in comparison to ProRoot MTA that does not contain a plasticizer. This fact highlights the importance of considering both the linear and volumetric flow for these materials. Similarly, Biodentine that contains polycarboxylate (as plasticizer) also had lower ISO flow in comparison to ProRoot MTA, but the highest cavity-filling among the tested materials, which corroborates with previous results [18]. Short initial setting time in balance with proper working

time for cement insertion is essential [11]. Biodentine and ProRoot MTA exhibited a shorter initial setting time in comparison to White-MTAFlow in 'thin' consistency. In future studies, the use of 'thick' or 'putty' consistencies would probably present different setting times for White-MTAFlow, once the powder to liquid ratio would be different from the one used in the present study.

An alkaline environment is essential to induce repair when hydraulic materials are used clinically [25]. All tested compositions showed alkaline pH regardless of the tested period. ProRoot MTA had a pH of around ten after 28-day immersion. Similar results for this material were shown by previous studies [26, 27]. Biodentine showed the lowest overall pH values, although significant increases were observed over time. White-MTAFlow showed comparable pH values after 24 hours and 28 days to those obtained for ProRoot MTA in this study.

Materials must present long-term volumetric stability to ensure its sealing ability against bacterial penetration [21]. The preferred time of analysis after 28 days was based in a previous report that highlighted this time as necessary to complete the hydration of these materials [6]. ProRoot MTA showed a slight beneficial increase in volume in our study, and a similar result was previously reported for MTA Angelus [28]. White-MTAFlow and Biodentine had a similar volumetric loss. Volumetric loss observed for Biodentine corroborates with previous findings [29]. In opposition to volumetric loss found for White-MTAFlow (9%), a previous study found a volume loss of 1.3% for Grey-MTAFlow using the same µCT methodology and the 'putty' consistency. This difference may be related to the White-MTAFlow different raw composition, the 'thin' consistency used in the present study, or the tantalum oxide radiopacifier used. Further studies with different consistencies are necessary to elucidate the reason for this higher volume loss demonstrated by the 'thin' White-MTAFlow.

Antimicrobial activity is expected from endodontic materials [21]. Regarding the antimicrobial activity of the tested materials, neither formulation satisfactorily inhibited bacterial growth in comparison to direct contact with chlorhexidine 2% in both methods. Previous studies showed similar results for ProRoot MTA [30] and Biodentine [31] using other methods. Solubility halos were observed over agar, showing that this initial test seems to be inadequate due to possible misleading interpretation. A previous study reported similar observations regarding Biodentine in contact with agar [32]. Direct contact with planktonic *E. faecalis* showed significantly higher values of turbidity and an apparent up-regulation of the bacterial growth for all the tested materials. Further studies with multispecies biofilms and live/dead bacterial quantification are undoubtedly necessary to clarify these findings.

*In vitro* cytotoxicity assays are preferred as initial testing for toxicity evaluation of new compositions [33] such as White-MTAFlow. This material had the lowest percentage of cell viability in our study. Biodentine, probably due to its composition and solubility, showed the highest cytocompatibility values corroborating with a previous study [20]. ProRoot MTA, as being the first commercially available material, studies had already proven its cytocompatibility [34], which corroborates with our findings. ProRoot MTA also exhibited previously reported comparable cytocompatibility results to those of Biodentine [35]. Future *in vivo* biocompatibility studies should be performed with White-MTAFlow in order to compare its results with the two other well-researched materials included in the present study.

## Conclusion

White-MTAFlow is a material comparable to ProRoot MTA and Biodentine. The use of this alternative radiopacifier does not cause color alteration in dentin and still provides acceptable values of radiopacity. Further studies evaluating the White-MTAFlow 'thick' and 'putty' consistencies could establish additional comparisons and are highly necessary to evaluate the biocompatibility of this new material.

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## Compliance with Ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical Approval** This study was approved by the Research Ethics Committee of the Piracicaba Dental School (Ethics code: CAAE 20189119.7.0000.5418).

**Informed consent** This study did not involve human subjects directly.

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**Figure legends**

**Fig. 1** X-ray diffraction plots of un-hydrated and hydrated cement and overlap diffraction. Calcium hydroxide (portlandite) was detected in hydrated samples of all tested compositions after 28-day immersion in Hank's Balanced Salt Solution (HBSS). Scanning electron micrographs (SEM) and energy dispersive spectroscopy (EDS). SEM micrographs and EDS plots of hydrated cement revealed particles of cement interposed by small particles of radiopacifier, with correspondent peaks detected. **a** Biodentine, **b** ProRoot MTA, and **c** White-MTAFlow

**Fig. 2 a d g** Representative specimens of bovine teeth filled with cement. Darkening is evident in ProRoot MTA cement with dentin presenting a dark color visible on the buccal surface, and staining in dentin is evident at the stereomicroscopic images of transversal cuts at 2x magnification. **b e h** Scanning electron micrographs and **c f i** elemental maps of sectioned teeth filled with the tested materials. Calcium (Ca), silicon (Si), and phosphorous (P) were found in all specimens. Bismuth (Bi) was found in ProRoot MTA cement, zirconium (Zr) was found in Biodentine, and tantalum (Ta) was found in White-MTAFlow corresponding to each material radiopacifier. The migration of radiopacifier and Si into the dentine was evident in the elemental maps. Bi was found to be high in the ProRoot cement matrix, with a high incidence of these ions at the cement/dentin interface. This was not verified in the White-MTAFlow, where Ta migration was reduced, and ions were more evenly distributed in the material matrix

**Fig. 3 a** Turbidity in contact with planktonic *E. faecalis* showing increased turbidity values after 48 hours (above graphic tendency bar). After 48 hours, negative control significantly reduced medium turbidity in comparison to positive control and tested cement. **b** Cytotoxicity graphic, in comparison to control (100%) Biodentine, had the highest cytocompatibility values in comparison to the other tested materials after 24 hours in contact with fibroblast cell culture. (#, *, §, +) Different symbols represent statistical differences between each evaluated period or tested material

**Table 1** Mean and standard deviation values of radiopacity, setting time, and flow. Different lowercase letters indicate statistically significant differences between the tested materials ($p < 0.05$)

| Material | Radiopacity | Setting time (min) | | Flow | | | |
|---|---|---|---|---|---|---|---|
| | (mm Al) | Initial | Final | ISO-6876:2012 (mm) | Linear lateral (mm) | Volumetric lateral (mm³) (max. 4 mm³) | Volumetric central cavity (mm³) (max. 2 mm³) |
| White-MTAFlow | 5.81 ± 0.79$^{ab}$ | 16.37 ± 0.90$^{a}$ | 47.67 ± 1.52$^{a}$ | 12.86 ± 1.34$^{a}$ | 5.56 ± 1.06$^{a}$ | 3.36 ± 0.23$^{a}$ | 1.45 ± 0.29$^{a}$ |
| ProRoot MTA | 6.38 ± 0.68$^{b}$ | 11.02 ± 0.58$^{b}$ | 29.67 ± 1.15$^{b}$ | 10.08 ± 1.04$^{b}$ | 5.28 ± 0.90$^{a}$ | 3.35 ± 0.32$^{a}$ | 1.52 ± 0.05$^{a}$ |
| Biodentine | 5.40 ± 0.18$^{a}$ | 11.80 ± 1.60$^{b}$ | 29.00 ± 2.00$^{b}$ | 8.26 ± 0.43$^{c}$ | 3.47 ± 0.85$^{b}$ | 2.62 ± 0.56$^{b}$ | 1.72 ± 0.28$^{a}$ |

**Table 2** Mean and standard deviation values of pH and volume change. Different letters in each column indicate statistically significant differences between tested periods (uppercase) and materials (lowercase) ($p < 0.05$)

| Composition | pH | | | Volume change* |
|---|---|---|---|---|
| | 3 hours | 24 hours | 28 days | (% after 28 days) |
| White-MTAFlow | 10.30 ± 0.14$^{Aa}$ | 9.65 ± 0.25$^{Ba}$ | 9.13 ± 0.24$^{Ba}$ | -9.15 ± 2.02$^{a}$ |
| ProRoot MTA | 9.92 ± 0.13$^{Ab}$ | 9.31 ± 0.50$^{Aa}$ | 9.55 ± 0.30$^{Aa}$ | +0.43 ± 0.76$^{b}$ |
| Biodentine | 7.84 ± 0.07$^{Ac}$ | 8.02 ± 0.03$^{Bb}$ | 8.43 ± 0.10$^{Cb}$ | -7.18 ± 0.33$^{a}$ |

*Volume change negative values represent volume loss, and positive values represent expansion

**Table 3** Mean and standard deviation values of color change (ΔE) after 90 days and lightness (L) at 24 hours, 28, and 90 days of analysis. Different lowercase letters indicate statistically significant differences ($p < 0.05$)

| Composition | Color change | Lightness | | |
|---|---|---|---|---|
| | 90d | 24h | 28d | 90d |
| Negative control | 6.35 ± 0.19[a] | 92.40 ± 1.15[a] | 90.73 ± 0.92[a] | 90.70 ± 0.26[a] |
| White-MTAFlow | 13.80 ± 3.20[bc] | 81.40 ± 1.65[b] | 90.83 ± 4.40[a] | 91.50 ± 4.25[a] |
| ProRoot MTA | 10.17 ± 0.75[b] | 81.56 ± 0.64[b] | 80.86 ± 0.30[b] | 78.06 ± 1.35[b] |
| Biodentine | 14.63 ± 0.83[c] | 88.13 ± 1.76[a] | 80.83 ± 1.62[b] | 82.20 ± 1.81[c] |
| Positive control | 289.13 ± 33.66[d] | 63.30 ± 4.48[c] | 44.76 ± 1.15[c] | 30.86 ± 0.60[d] |

Figure 1



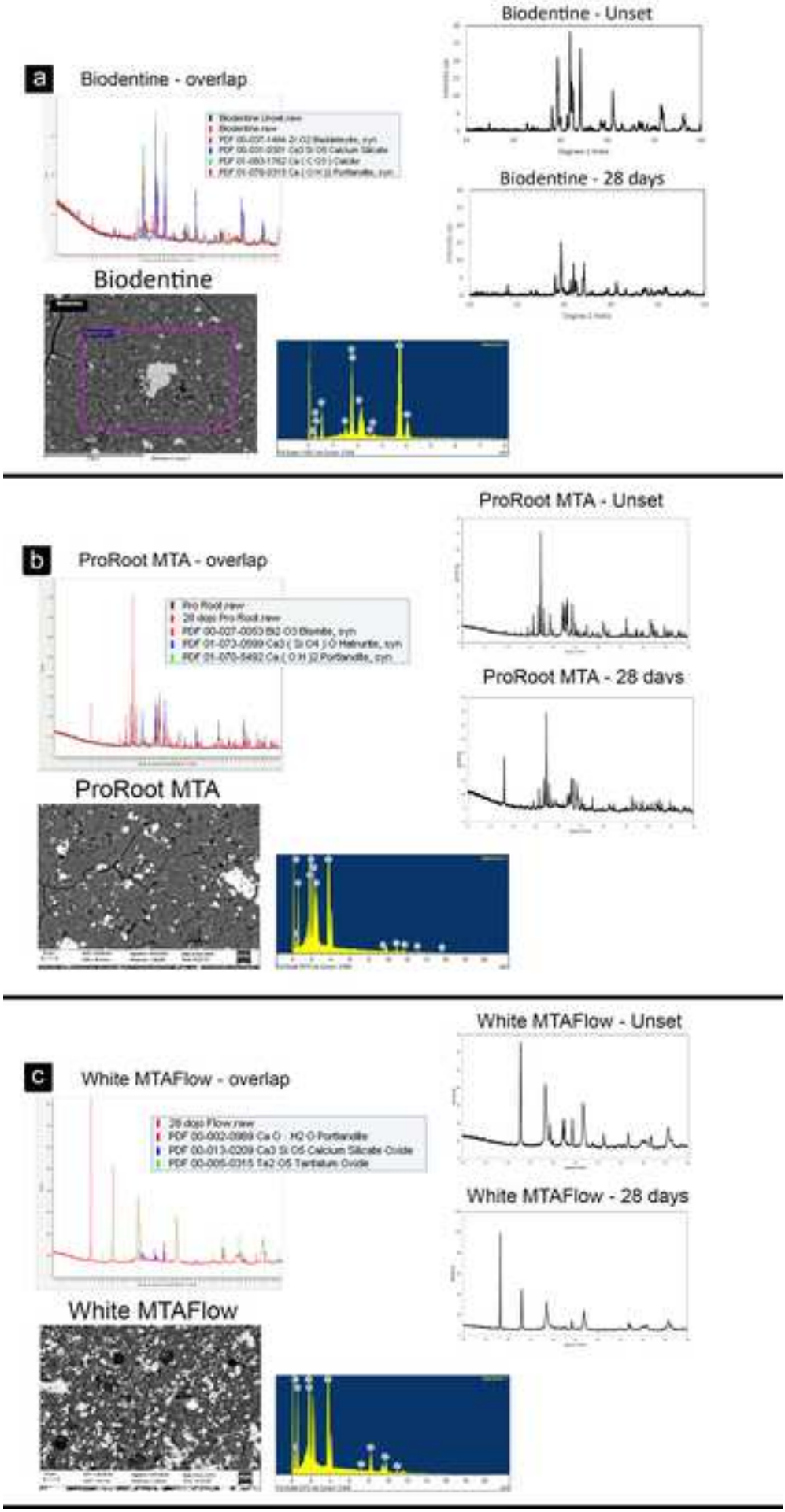

Figure 2

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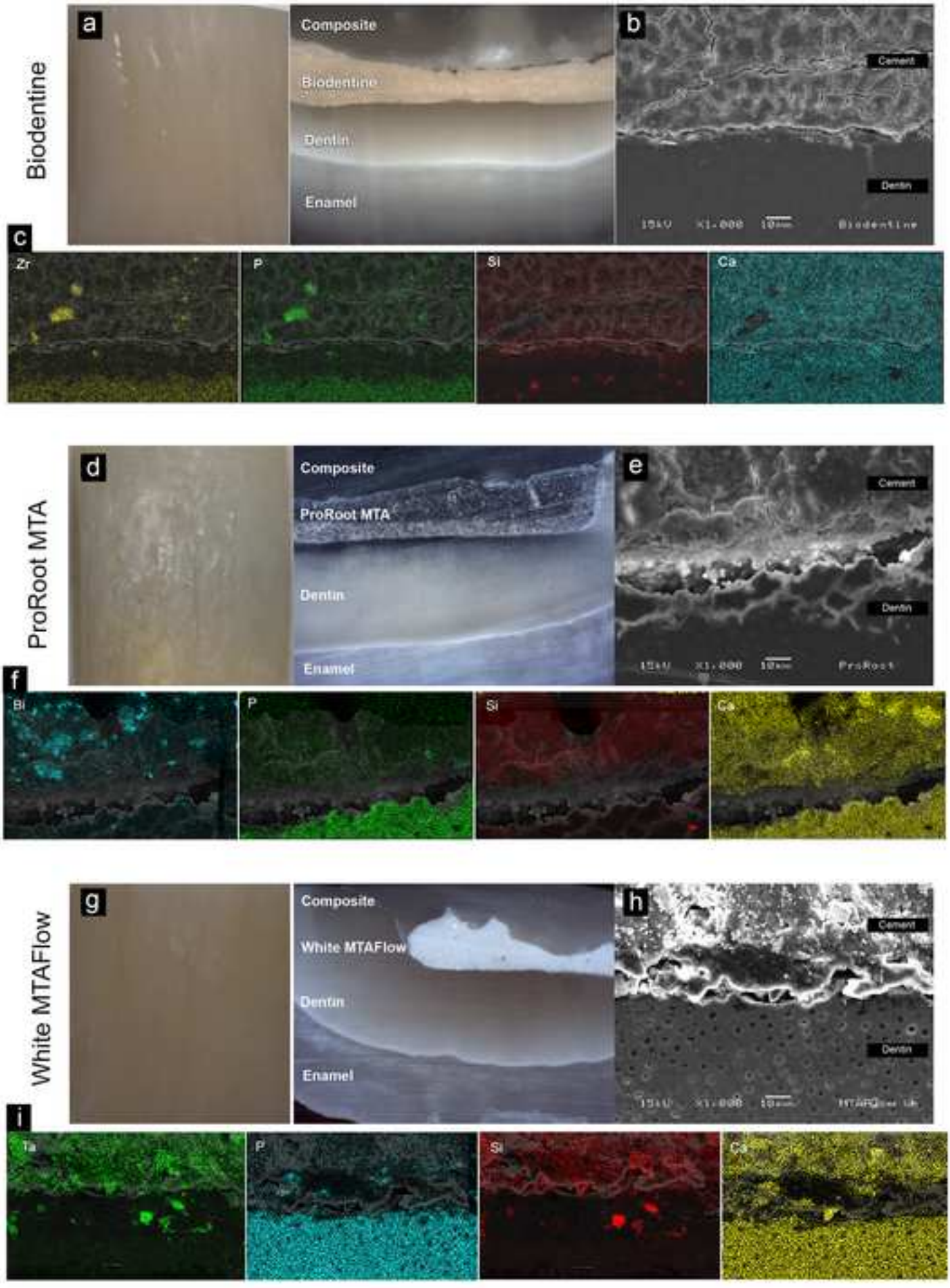

Figure 3