Influence of contact with blood and saline on solubility, pH and chemical composition of MTA

Lyz Cristina Furquim **CANALI**[1]
Carlos Alberto Herrero de **MORAIS**[2]
Bruno Cavalini **CAVENAGO**[3]
Rodrigo Ricci **VIVAN**[4]
Marco Antonio Hungaro **DUARTE**[5]

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ABSTRACT

Objective: The aim of this study was to evaluated the solubility, pH level and chemical constitution of white MTA in contact with blood and saline solution. **Methods:** Thirty acrylic teeth replicas of a maxillary central incisor, with 3 mm root-end cavity were filled with white MTA. The samples were inserted individually in Eppendorf microtubes and divided into 3 groups (n = 10). In the Group 1 the specimens were not exposed to liquids (control group). In the Group 2 microtubes received 1 mL of saline solution and Group 3 received 1 mL of fresh human blood. The specimens were stored at 37 °C for 24 hours. The solubility was volumetric evaluated so the specimens were scanned by a Micro-CT and individually immersed in 10 mL of ultrapure water, after 168 hours new scans were performed. The pH level was measured after 168 hours. Sections of 2 mm thickness were analyzed using energy dispersive spectroscopy to evaluate the ionic composition. The data were statistically analyzed by the ANOVA and Tukey tests. **Results:** The solubility was similar ($p > 0.05$) for all groups. After 168 hours the pH was significantly higher for Group 2 in comparison with Group 3 ($p < 0.05$). In the ionic composition was found higher concentration of calcium and the contact with blood did not interfere on ion calcium release. **Conclusion:** The contact of MTA with blood and saline solution did not influence in the solubility, both favored an alkaline pH with increased of ion calcium release with small variations in the ionic composition.

Keywords: Endodontics; Dental materials. Materials testing. X-ray microtomography. Scanning electron microscopy.

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[1]PhD Student of Endodontics, Dental School of Bauru, University of São Paulo.

[2]Associate Professor of Endodontics, State University of Maringá.

[3]Adjunct Professor of the Restorative Dentistry Department, Federal University of Paraná.

[4]PhD Professor of Dentistry, Endodontics and Dental Materials of the Dental School of Bauru, University of São Paulo.

[5]Associate Professor of the Department of Dentistry, Endodontics and Dental Materials of the Dental School of Bauru, University of São Paulo.

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Contact address: Bruno Cavalini Cavenago
Av. Prefeito Lothário Meissner, 3.400 - C.P.: 2558
CEP: 80.210-170
E-mail: brunocavenago@ufpr.br

Introduction

MTA is a suitable material for retrograde filling because meets the most of the requirements for an ideal cement for this purpose with good physical and chemical characteristics[1,2] and excellent biological properties.[3,4,5,6] The solubility of retrofilling cement is undesirable because the material dissolving could permit the bacterial infiltration and compromise the quality of the treatment. The MTA has low solubility detected both by conventional methodologies based on weight[7,8,9] and by tridimensional volumetric analyses.[10]

The ability to ions calcium release and maintain the alkaline medium are important chemical properties, because these conditions could promote the repair by stimulating the mineralization process.[11] The setting time of MTA occurs around 165 minutes[1] and also able to promote the alkaline pH and ion calcium release.[7,8]

In endodontic surgery, pulpotomy or root perforation sealing with MTA can occur the contact of cement with blood and tissue fluids. Blood in contact with the surface becomes incorporated into the MTA, but few studies have examined this association, which can influence the compressive strength and cement surface microstructure.[12] However other physical and chemical properties of white MTA in contact with blood were still unclear. Therefore the aim of this study was to evaluate the solubility, pH and ionic composition of white MTA in contact with blood and saline solution.

Materials and Methods

This study was approved by Human Research Ethics Committee of the State University of Maringá (CAAE - 28585814.4.0000.0104). The white MTA cement (Angelus, Londrina, PR, Brazil) was mixed using 3 parts of powder and 1 part of distilled water. Thirty acrylic teeth replicas of a maxillary central incisor, with standardized root-end cavity with 3 mm deep were filled with cements using a MTA carrier device. The specimens were visually inspected with 5x magnifying glass to ensure that not remain voids or gaps. Thirty Eppendorf microtubes were prepared by inserting a cotton pellet followed by a fragment of hemostatic sponge with lyophilized hydrolyzed collagen (Hemospon, Technew, Rio de Janeiro, Brazil) to simulate the periodontal ligament. Each sample was inserted individually in Eppendorf microtubes and divided into 3 groups (n = 10).

In Group 1, the specimens were inserted in microtubes without liquids (control group). In Group, 2 microtubes received 1 mL of saline solution and Group 3 received 1 mL of fresh human blood collected from a healthy volunteer member of the research group. The specimens were incubated at 37 °C for 24 hours.

Solubility and pH evaluations

The solubility was volumetrically measured using microcomputed tomography images (10). The samples were scanned using a desktop X-ray microfocus CT scanner (SkyScan 1174v2; SkyScan, Kontich, Belgium). The scanning parameters was determined using 50 kV X-ray tube voltages, 800 μA anode current, a voxel size of 14.1 μm, with 1.1° rotation step and 360° rotation. Digital data with 1024 x 1304 pixels were elaborated by reconstruction software (NRecon v. 1.6.4.8, SkyScan), and the CTan software (CTan v. 1.11.10.0, SkyScan) was used for the volume measurements. For each sample a binary value was adjusted and through the 3D plug-in analysis the total volume (mm^3) was recorded.

After the first scanning the samples were individually immersed in glass flasks containing 15 mL of ultrapure water with the pH level previously measured, and then were stored at 37 °C for 168 hours. Thereafter, the samples were scanned one more time using exactly the same parameters adopted for the first scan, as well as the volume analysis. So the results found were converted to percentages of solubility.

The measurement of the pH level of the water in glass flasks was carried out with a calibrated pH meter (model 371; Micronal, São Paulo, Brazil), after 24 and 168 hours of the specimens immersion.

Determination of ionic composition by energy dispersive spectroscopy (EDS)

The evaluation of the ionic composition was performed by making a section with 2 mm in thickness in the final segment of specimens. The surface of a specimen of each group was evaluated with the

Figure 1. Micro-CT tridimensional reconstructions of the representative samples. Group 1 (**A** and **B**), Group 2 (**C** and **D**) and Group 3 (**E** and **F**), before and after water immersion, respectively.

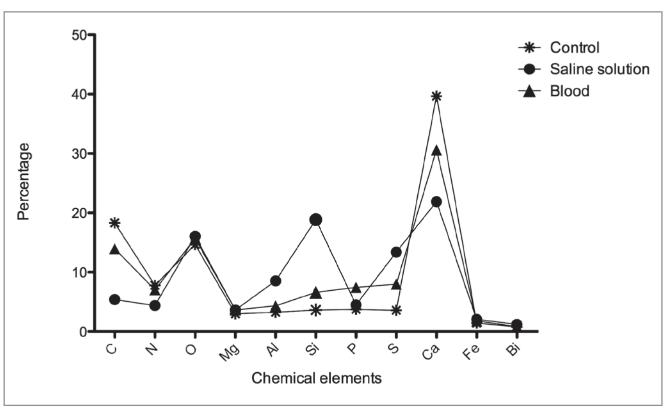

Figure 2. Graphic representation of percentage of chemical elements showed by EDS.

personal scanning electron microscopy (PSEM) (Aspex Express; Fei Europe, Eindhoven, Netherlands) at an accelerating voltage of 15–20 kV. The elemental composition analysis was conducted by energy dispersive spectroscopy (EDS), which is fully integrated to the PSEM.

Statistical analysis

The solubility and pH level were evaluated by the ANOVA and Tukey's tests. The significance level was set at 5% and the Prism 5.0 software (GraphPad Software Inc, La Jolla, CA, USA) was used as the analytical tool.

Results

In all groups the solubility was less than 3%, and was no statistically significant difference between groups ($p > 0.05$). The results of solubility are showed in Table 1. Figure 1 shows the representative micro-CT three-dimensional reconstructions of first and final scannings.

The pH level in the control group was significantly lower ($p < 0.05$) than the experimental groups. But in the groups with exposure to saline solution and blood had similar pH. Table 2 contains the pH data.

In qualitative analysis of the ionic composition by EDS, were observed the presence of chemical elements such as carbon (C), nitrogen (N), oxygen (O), magnesium (Mg), aluminum (Al), silicon (Si), phosphorus (P), sulfur (S), calcium (Ca), iron (Fe), bismuth (Bi), such as the composition of MTA Angelus®. In Group 1 was found the largest volume of Ca (42.8%). In Group 2 the percentage of Ca was 19.1%, whereas in Group 3 was 33.4%.

Table 1. Mean and standard deviation of solubility (%) and the pH values found after 168 hours. Different letters in each column indicates statistically differences ($p < 0.05$).

Group	Solubility	pH
G1 – control group	2.01 (1.68)[a]	7.91 (0.07)[ab]
G2 – saline solution	2.16 (1.16)[a]	7.99 (0.11)[a]
G3 – blood	1.91 (0.91)[a]	7.82 (0.17)[b]

Discussion

The MTA is a cement that was developed in the 1990s, indicated as retrofilling material and for root perforations sealing.[13,14] Since then, several studies have been conducted to clarify their physical, chemical and biological properties.[2,5] However some factors such as the influence of blood on some physicochemical properties are still unclear. Therefore this study aimed to evaluate the effect of contact with blood in solubility, pH level and ionic composition of white MTA.

Usually *in vitro* tests evaluate the solubility property following the specifications of ANSI/ADA #57 or ISO 6876, these standards are based on the difference between weights before and after placing the cement into distilled water.[1,8,9,15] However, this study follow the methodology based on volumetric change of three-dimensional images obtained from scanning of the specimens by micro-CT,[10] this methodology has great advantage because it evaluates only the radiopaque material, excluding the water in analysis — this is a factor that should be considered because the MTA is a hydrophilic cement. Also, were used specimens that simulates the clinical aspect in better form, and allowing to compare the data with other studies that used the cement in retrofilling cavities in acrylic replicas of natural teeth.[7,10,16]

The solubility is a property directly related to the dissociation of the constituents of the material by contact action with the surrounding liquid, with result in material dissolution. Thus, the larger the contact area, as found in specimens according ISO standard, the greater is the possibility of higher solubility. The solubility was similar in the different groups of this study. It could be considered acceptable because they were below 3%, corroborating with other studies evaluating this cement,[7,10,17] but the important fact revealed by this study is that the blood did not affect this property.

All cements showed an alkaline pH regardless of the evaluated period, and this result is in agreement with the specific literature.[2,8,15,17,18] The groups where MTA was exposed to saline solution or blood showed higher pH when compared to the control group, probably it was due to greater moisture in the experimental groups, due to exposure to fluids that favored increased ion calcium release. This factor is directly related to the pH provided by the cement.[8]

The characterization of endodontic cements can be performed by using SEM-EDS analysis. It is a certified method for evaluating the ionic composition.[19,20] In the control group was found the largest amount of Ca ions. Probably because it was not exposed to contact with liquids, there was no ionic dissociation with the environment. On the other hand, in Group 2 was found the least amount of Ca, precisely because it was exposed to contact with saline solution, it increased the solubility and ion calcium release.

The radiopacifier of white MTA Angelus could change the color of cement. According to Marciano et al,[21] bismuth oxide reacts with collagen of dental structure, resulting in a grayish discoloration of cement. Furthermore, the MTA contact with blood promote color alteration due the oxidation of the heme group present in hemoglobin and interfere in the radiopacity over time.[22] However, these conditions are not critical when the cement is used for retrofilling, and the results of this study showed similar solubility, acceptable pH level and ion composition of cement.

Conclusion

The contact of MTA with blood and saline solution did not influence the solubility, both favored an alkaline pH with increased ion calcium release and small variations in the ionic composition.

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