Radiopacity, pH and antimicrobial activity of Portland cement associated with micro- and nanoparticles of zirconium oxide and niobium oxide

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The aim of this study was to evaluate some properties of the calcium silicate materials Mineral Trioxide Aggregate (MTA) and Portland cement (PC) with microparticulated (micro) and nanoparticulated (nano) zirconium oxide (ZrO2) or niobium oxide (Nb2O5). The experimental materials: White PC (PC), MTA-Angelus® (MTA), PC+ZrO2 micro, PC+ZrO2 nano, PC+Nb2O5 micro and PC+Nb2O5 nano were submitted to radiopacity and pH evaluations. Furthermore, the antimicrobial activity against different microorganisms was assessed by agar diffusion test. MTA presented higher radiopacity than other materials. However, all materials except PC presented higher radiopacity than recommended by ISO/ADA. MTA promoted higher pH values in all analyzed periods (p<0.05). At the initial periods, PC and PC+ZrO2 micro showed pH similar to MTA. All materials showed antimicrobial activity against the evaluated microorganisms. In conclusion, ZrO2 and Nb2O5 could be alternative radiopacifiers to be added to calcium silicate materials.

Keywords: Mineral Trioxide Aggregate, Root-end filling material, Portland cement

INTRODUCTION

Mineral Trioxide Aggregate (MTA) is a dental material widely used for root perforation repair, retrograde filling and teeth with open apices5. MTA has proper physicochemical properties, sealing capacity, biocompatibility, and ability to induce bone mineralization2-4. MTA is basically composed by Portland cement (PC) which has physicochemical, antimicrobial and biological properties similar to commercially available MTA5-30. PC shows biocompatibility and high compressive strength allowing that this material may be suitable for medical indications, such as orthopedic applications5.

Bismuth oxide (BO) is the radiopacifier agent for MTA7. However, it is related that this substance interferes with the hydration mechanism of MTA and calcium hydroxide precipitation in the hydrated cement7. Furthermore, the association of BO changes the microstructure of the cement matrix8, and increases the porosity and solubility of the Portland cement reducing its resistance8, 9.

Other materials have been evaluated as alternative to bismuth oxide, such as, zirconium oxide (ZrO2) which provides proper radiopacity10,11, and cell viability12. Also, ZrO2 associated with PC presents radiopacity, compressive strength, setting time, water absorption and solubility similar to ProRoot MTA13. Tanomaru-Filho et al.14 evaluated the compressive strength and setting time of MTA and PC associated with bismuth oxide, ZrO2, calcium tungstate, showing that all radiopacifying agents may be used in association with PC to replace bismuth oxide. Camilleri et al.15 reported that the hydration reaction of 30% of ZrO2 added to PC produces calcium silicate hydrate, calcium hydroxide and a minimum amount of monosulfate and ettringite (hydrous calcium aluminum sulfate mineral) and, thus, does not affect the hydration mechanism.

Niobium oxide (Nb2O5) has been studied to enhance mechanical properties in titanium alloys of osseointegrated implants due to its excellent biocompatibility and resistance to corrosion16,17. In dentistry, Nb2O5 has been evaluated as radiopacifying agent of dental materials, such as, root canal sealers, promoting proper physicochemical properties17.

It has been suggested that the use of nanoparticles could improve the physicochemical properties of MTA18. It was already demonstrated that a MTA-based material composed by small particles shows reduced setting time and increased microhardness18. When added to PC, ZrO2 nanoparticles change the microstructure of the material during the hydration reaction, improving cellular proliferation and adhesion19. Nb2O5 nanoparticles demonstrated bioactivity and biocompatibility20 and the capacity to increase material resistance21. Besides, the use of nanoparticles may increase the antibacterial activity of different materials22.

Therefore, once the association of nanoparticulated ZrO2 or Nb2O5 can improve physicochemical and antimicrobial properties of PC, the aim of this study was to evaluate radiopacity, pH and antimicrobial activity of these materials.

MATERIALS AND METHODS

Six experimental groups were established according the Table 1. A ratio of 30% ZrO2 or 30% Nb2O5 and 70% PC by weight were used for materials preparation. The PC+ZrO2 nano and PC+Nb2O5 nano were manipulated by
Table 1  Composition and manufacturers of the materials evaluated

<table>
<thead>
<tr>
<th>Materials</th>
<th>Compositions</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>White Portland cement</td>
<td>Tricalcium silicate, dicalcium silicate, calcium aluminate, calcium sulfate, tricalcium aluminate, calcium carbonate, magnesium oxide, calcium oxide</td>
<td>WPC-40 structural Votoran, Votorantin cements, Brazil</td>
</tr>
<tr>
<td>MTA-Angelus*</td>
<td>80% Portland cement* 20% Bismuth oxide*</td>
<td>Angelus, Londrina PR, Brazil</td>
</tr>
<tr>
<td>PC+ZrO₂micro</td>
<td>70% Portland cement* 30% Zirconium oxide microparticulated*</td>
<td>Sigma-Aldrich Brasil Ltd., São Paulo – SP, Brazil</td>
</tr>
<tr>
<td>PC+ZrO₂nano</td>
<td>70% Portland cement* 30% Zirconium oxide nanoparticulated*</td>
<td>Laboratory of Nanotechnology, Institute of Physics of de São Carlos, SP, Brazil</td>
</tr>
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* by weight

using a powder:liquid ratio of 1 g:0.33 mL, standardized in previous tests, providing proper handling and consistency for a root-end filling material. The other materials were mixed at a ratio of 1 g powder of cement per 0.3 mL liquid (distilled water). Nanoparticulated radiopacifiers were performed by polymeric precursor method at Institute of Physics of São Carlos (University of São Paulo, São Carlos, Brazil) as described by Viapiana et al.\(^1\). The particle size obtained for ZrO\(_2\) was 74 nm and for Nb\(_2\)O\(_5\) was 83 nm which was confirmed by Brunauer-Emmett-Teller (BET) surface area analysis.

**Radiopacity**

Six samples of each material were submitted to the radiopacity test, which was performed according to Tanomaru-Filho et al.\(^2\). The samples were made using plastic rings (internal diameter=10 mm; height=1 mm). The specimens were stored at 37°C and 100% of humidity for 48 h until setting. After this period, specimens were placed on five occlusal radiographic films (Insight-Kodak Comp, Rochester, NY, USA) and exposed, along with an aluminum step wedge with variable thickness (from 2 to 16 mm, in 2-mm increments). The radiographs were taken using an X-ray device (GE 1000, General Electric, Milwaukee, WI) at 50 kV, 10 mA and 18 pulses per second, with focal distance of 33 cm. The films were processed in an automatic radiographic processing machine and digitized. The images were assessed using the Image Tool software (UTHSCSA Image Tool for Windows version 3.0); an equal-density tool was used to identify equal-density areas in the radiographic images. This procedure allowed comparison between the radiographic density of the materials and the radiopacity of the different aluminum step wedge thicknesses. The area corresponding to the specimen was selected in each radiographic image to verify the thickness of the aluminum step wedge detected by the software as equivalent to the material’s radiographic density. Thus, the radiopacity of the evaluated materials was estimated using a conversion equation\(^10\). The values recorded for each material were averaged to obtain a single value in mmAl.

**pH**

To perform the pH test, 10 standardized polyethylene tubes, measuring 10.0 mm length and 1.6 mm diameter, were filled with each material. Then, each tube was immersed into 10 mL of distilled water and stored at 37°C during the experimental period. After 1 day of immersion, the tubes were carefully removed and placed into new flasks with an equal amount of distilled water, and this procedure was repeated after 3, 7, 14, 21 and 28 days. The solutions pH was analyzed at each period using a calibrated digital pH meter (Ultrabasic; Denver Instrument Company, Arvada, CO).

**Antimicrobial activity**

The materials were submitted to antimicrobial activity test using agar diffusion test. Table 2 shows the strains used for antimicrobial evaluation. All strains were used from American Type Culture Collection (ATCC). The inoculum was obtained in MHB (Mueller Hinton Broth – Difco) using incubation at 37°C for 24 h. The tests using well-diffusion method were performed in triplicate, in double-layer plates, with a base and a seed layer. The base layer was obtained with 15 mL of sterilized culture medium plated onto sterilized Petri plates (20×150 mm). After solidification, 8 mL of seed layer was added, obtained with the addition of the inoculum to the culture medium. A final concentration of 10⁶ CFU/mL was calibrated by spectrophotometer.
Table 2 Strains used as indicators for antimicrobial activity

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Origin</th>
</tr>
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<tbody>
<tr>
<td>Kocuria rhizophila</td>
<td>ATCC 9341</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>ATCC 25175</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>ATCC 29212</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 27853</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC 10231</td>
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</table>

After the seed layer solidification, wells were obtained removing the agar by using sterilized metallic tubes (internal diameter=4 mm) at equidistant points. The material were prepared and inserted into the wells. The plates were kept at environmental temperature (25±1°C) for two hours, to material pre-diffusion and then plates were incubated at 37°C for 24 h. After the incubation, aliquots of 5 mL of TTC gel (prepared with 1.0% agar and 0.05% of triphenyltetrazolium chloride) were added to optimize the evaluation. After solidification the samples were incubated at 37°C for 30 min.

The images of the plates were digitized and the diameter of inhibition halos around the wells were measured using the Image Tool software (UTHSCSA Image Tool for Windows version 3.00).

Statistical analysis
The obtained data for radiopacity and pH tests were submitted to ANOVA and Tukey test. Data from antimicrobial activity were submitted to Kruskall Wallis and Dunn tests (p≤0.05).

RESULTS

Radiopacity
PC showed less radiopacity than all the other materials. MTA radiopacity values were greater than all groups. However, the associations of PC with micro or nano ZrO₂ and Nb₂O₅ showed radiopacity above to the minimum recommended by ISO/ADA of 3.0 mmAl (Table 3).

pH
The evaluated materials promoted alkalinity in all analyzed periods. After 1 day, it was observed a higher pH for MTA. In the periods of 1 and 3 days PC, PC+ZrO₂ micro and MTA showed similar pH. At other periods, MTA had higher pH than the evaluated materials (p≤0.05) (Table 4).

Antimicrobial activity
All materials showed similar antimicrobial activity against the bacterial strains evaluated.

DISCUSSION
Radiopacity is important for an endodontic material to be distinguished from the surrounding structures, such as dentine and alveolar bone. The method using the aluminum scale is recommended by ANSI/ADA standards, which established a minimum corresponding to 3 mmAl for an endodontic material.

PC presents radiopacity less than 2 mmAl, and is...
not in accordance with American Dental Association - ANSI/ADA\textsuperscript{20}. MTA is a biocompatible material composed by PC added to 20% of bismuth oxide as radiopacifier\textsuperscript{20}. In the present study, MTA showed 5 mmAl as radiopacity value, in accordance with previous studies\textsuperscript{10}. MTA’s radiopacifier is bismuth oxide, which promotes radiopacity when associated with PC\textsuperscript{10,11}. Notwithstanding, bismuth oxide may increase PC’s porosity, damaging the longevity of the material\textsuperscript{7,8}. The results of the present study showed that the addition of ZrO\textsubscript{2} micro to PC promotes proper radiopacity, as showed by Húngaro Duarte\textsuperscript{10} and Bortoluzzi et al.\textsuperscript{11}. Besides, the association of NbO\textsubscript{5} with PC showed radiopacity in accordance with American Dental Association - ANSI/ADA.

The antimicrobial activity evaluation showed similar results for all the microorganisms evaluated. The addition of substances to MTA aiming to improve its antimicrobial effect has been studied\textsuperscript{27-29}. Odabas et al.\textsuperscript{28} demonstrated that silver addition to MTA improves its antimicrobial property. Saatchi\textsuperscript{25} et al. observed that the inclusion of potassium iodide does not alter the antimicrobial effect of MTA. Guerreiro-Tanomaru et al.\textsuperscript{28} showed that Portland cement-based materials with the addition of different radiopacifiers (bismuth oxide, calcium tungstate, and zirconium oxide) presented antimicrobial activity and pH similar to pure PC. The alkaline pH of MTA may be important for the calcium carbonate formation, which could contribute to the sealing ability of MTA\textsuperscript{30}. Coronal leakage after sealing with MTA was lower than dentin-adhesive materials and silicon-based sealer\textsuperscript{31}. The high pH value of MTA is attributed to the constant calcium release and calcium hydroxide formation. The antifungal effect of MTA might be due to its high pH and also to substances released from MTA\textsuperscript{32}.

In the present study, the addition of ZrO\textsubscript{2} and NbO\textsubscript{5} micro and nanoparticulated did not influence on the antimicrobial effect of PC. The results of the pH assessment showed that MTA exhibited the highest pH when compared to the other materials. All cements showed an alkaline pH, indicating the capacity of these materials to release hydroxyl ions. Massi et al.\textsuperscript{32} reported similar pH results for both PC and MTA. The high pH of MTA can be attributed to the higher solubility of this material. According to Coomaraswamy et al.\textsuperscript{8}, the association of bismuth oxide with PC results in failures in the cement matrix which increases the porosity of the material, leading to a greater solubility and degradation.

The associations of ZrO\textsubscript{2} and NbO\textsubscript{5} micro and nano with PC showed proper results for all tests performed in the present study. It has already been demonstrated that the association of ZrO\textsubscript{2} with PC promotes radiopacity\textsuperscript{10,11}, not causing damage to the fibroblasts\textsuperscript{4} and periodontal ligament cells\textsuperscript{32}. It was also showed that 30% of ZrO\textsubscript{2} added to PC does not interfere in the hydration mechanism of this cement, providing calcium ions release and resulting in a bioactive material\textsuperscript{10}. Additionally, this association exhibits proper physicochemical properties, similar to MTA\textsuperscript{10}. Li et al.\textsuperscript{30} showed that nanoparticulated ZrO\textsubscript{2} particles provides efficient nucleation for the precipitation and growth of the C-S-H gel phase which increase the degree of material hydration.

NbO\textsubscript{5} has been indicated for the treatment of implant surfaces, due to its capacity to favor the osseointegration\textsuperscript{38}. The use of niobium oxide as a radiopacifying agent of dental materials has also been studied, resulting in proper physicochemical properties, increasing radiopacity and microhardness\textsuperscript{31}. The obtained results showed that micro and nano NbO\textsubscript{5} can be considered as potential radiopacifiers in association to PC, promoting radiopacity, alkaline pH and antimicrobial activity. However, further studies are necessary to evaluate other physicochemical properties as well the biological behavior of these materials.

**CONCLUSION**

Considering the methodology used and the obtained results, it can be concluded that ZrO\textsubscript{2} and NbO\textsubscript{5} micro and nanoparticulated may potentially be used as radiopacifying agents.

**ACKNOWLEDGMENT**

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