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## BASIC RESEARCH

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Propiedades físico-químicas y actividad antibiofilm del cemento de silicato tricálcico y su asociación con cetrimida

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**ABSTRACT:** Cetrimide (CTR) is a cationic surfactant detergent with antimicrobial and antibiofilm activity. The aim of this study was to evaluate setting time, pH, solubility and antibiofilm activity of tricalcium silicate cement (TSC) with zirconium oxide (ZrO<sub>2</sub>) and its association with 0.2 and 0.4% cetrimide. Initial and final setting times (IST and FST) were assessed based on ISO-6876. pH was evaluated at periods of 1, 3, 7, 14 and 21 days. Solubility was analyzed by weight loss. A modified direct contact test (MDCT) on the biofilm of *Enterococcus faecalis* formed on bovine root dentin blocks was performed, after 6 hours of manipulation and 15 hours of contact time. The analysis was performed by UFC mL<sup>-1</sup> counting. The data were analyzed by ANOVA and Tukey's tests ( $\alpha=0.05$ ). Higher IST was observed for TSC/ZrO<sub>2</sub>+CTR in both concentrations than for TSC/ZrO<sub>2</sub> and lower FST for TSC/ZrO<sub>2</sub>+0.4% CTR ( $p<0.05$ ). On day 1, TSC/ZrO<sub>2</sub> showed lower pH than the associations with CTR ( $p<0.05$ ). During the other periods, TSC/ZrO<sub>2</sub> and associations promoted similar alkalization ( $p>0.05$ ). All materials exhibited increased mass. TSC/ZrO<sub>2</sub>+CTR 0.4% had lower mass gain than the other materials ( $p<0.05$ ). The highest antibiofilm activity was observed for

TSC/ZrO<sub>2</sub>+CTR in both concentrations, when compared with the positive control ( $p<0.05$ ). In conclusion, CTR exhibited potential to promote greater antibiofilm activity to tricalcium silicate cement, without harming its physicochemical properties of setting time, pH and solubility.

**KEYWORDS:** Silicate cement; Physicochemical; Antibacterial; Endodontics; Biofilms; Dental materials.

**RESUMEN:** Cetrimida (CTR) es un detergente y surfactante catiónico con actividad antimicrobiana y antibiofilm. El objetivo de este estudio fue evaluar el tiempo de fraguado, pH, solubilidad y actividad antibiofilm del cemento de silicato tricálcico (CST) con óxido de zirconio (ZrO<sub>2</sub>) y su asociación con CTR a 0.2% y 0.4%. Tiempo de fraguado inicial y final (TFI y TFF) fueron determinados con base en las normas ISO-6876. pH fue evaluado en los períodos de 1,3, 7,14 y 21 días. Solubilidad fue analizada por la pérdida de masa/peso. El test de contacto directo modificado (TCDM) fue realizado sobre biofilm de *Enterococcus faecalis* formado en dentina radicular bovina, después de 6 horas de manipulación de los cementos y 15 horas de contacto. El análisis fue realizado por la cuantificación de UFC mL<sup>-1</sup>. Los datos fueron analizados usando las pruebas de ANOVA y Tukey ( $\alpha=0.05$ ). Mayor TFI fue observado para CST/ZrO<sub>2</sub>+CTR en las dos concentraciones que para TSC/ZrO<sub>2</sub> y menor TFF para TSC/ZrO<sub>2</sub>+CTR 0.4% ( $p<0.05$ ). En 1 día, TSC/ZrO<sub>2</sub> mostró menor pH que las asociaciones con CTR ( $p<0.05$ ). En los otros períodos, TSC/ZrO<sub>2</sub> y las asociaciones promovieron alcalinización de forma similar ( $p>0.05$ ). Todos los materiales mostraron aumento de masa. TSC/ZrO<sub>2</sub>+CTR 0.4% tuvo menor ganancia de masa que los otros materiales ( $p<0.05$ ). Mayor actividad antibiofilm fue observado para CST/ZrO<sub>2</sub>+CTR en las dos concentraciones, cuando comparados con el grupo control positivo ( $p<0.05$ ). En conclusión, CTR demostró potencial para promover superior actividad antibiofilm al cemento de silicato tricálcico (CST), sin perjudicar sus propiedades físico-químicas de tiempo de fraguado, pH y solubilidad.

**PALABRAS CLAVE:** Cemento de silicato; Físico-química; Antibacteriano; Endodoncia; Biofilms; Materiales dentales.

## INTRODUCTION

Mineral Trioxide Aggregate (MTA) is a biocompatible repair material (1) that contains calcium silicates and bismuth oxide as radiopacifying agents (2). MTA stimulates mineralization and provides alkaline pH; in addition to present sealing capacity (3-5). Endodontic repair materials are used to promote repair by mineralized tissues (6,7). MTA presents some disadvantages, such as possibility of containing heavy metals, and limited

antimicrobial activity (8-12). The radiopacifying agent bismuth oxide may affect some of its physicochemical properties and promotes tooth discoloration (11,13,14).

Tricalcium silicate is the main component of MTA and is used to development of reparative materials (2-4,14,15). Pure tricalcium silicate cement (TSC) is free of heavy metals, which may be present in Portland cement (10,11,14,15). These materials have demonstrated properties similar

to MTA (3,9,10), such as cytocompatibility (16), bioactivity (17), and induction of cell differentiation (15). Experimental TSC have shown proper hydration, high pH, calcium ion release, water absorption and porosity similar to MTA (18).

Repair materials must have proper radiopacity to be distinguished from the adjacent structures (10). TSC have lower radiopacity than recommended by ISO-6876 (10,19). The association of 30% ZrO<sub>2</sub> with TSC promotes proper radiopacity and biocompatibility (9,20).

Successful treatment is directly related to control of infection present in the root canal system (RCS). Therefore, it is desirable for repair materials to have antimicrobial activity (8,9). Cetrimide (CTR) is a cationic surfactant with effective antimicrobial and antibiofilm activity against *Enterococcus faecalis* (21,22). In addition to eradicating *E. faecalis in vitro*, CTR reduces the surface tension of chelating solutions, and is responsible for enhancing antimicrobial effectiveness (22-24). Therefore, the incorporation of surfactants into endodontic materials has been reported (22,25-27), and it has demonstrated the potential to improve the antimicrobial activity (21,22).

The aim of this study was to evaluate the physicochemical properties and antibiofilm activity of TSC (70%) with ZrO<sub>2</sub> (30%) and their association with CTR (0.2% and 0.4%). The null hypothesis of this study was that CTR would not interfere in the physicochemical properties and antibiofilm activity of TSC associated with ZrO<sub>2</sub>.

## MATERIAL AND METHODS

The materials were divided into 3 experimental groups (Table 1). The materials were manipulated in the proportion of 1 g of powder/360 µL of liquid (distilled water or CTR in water solution -5,5mg/mL ou 11mg/mL).

### SETTING TIME

The setting time (ST) was evaluated according to ISO-6876:2012 (19). Metal rings 10 mm in diameter by 1mm high were used (n=5). The cements were manipulated and inserted inside the metal rings at 37 °C and air humidity of 95%. To determine the initial setting time (IST) a Gilmore needle with 100±0.5 g and tip diameter 2±0.1mm was used. To evaluate the final setting time (FST), the same procedure was repeated, using Gilmore needle with 456±0.5g and tip diameter 1±0.1mm. The IST and FST (min) were determined as the periods between manipulation and the time at which each needle no longer produced marks on the cements.

### PH

Polyethylene tubes (length of 10mm and an internal diameter of 1.6mm) were used (n=10). The cements were inserted inside the tubes and each specimen was placed in a plastic flask containing 10 mL deionized water and stored at 37°C. The pH measurements were performed after 1,3,7,14 and 21 days of immersion, using a digital pH meter (Digimed DM-22, SP, Brazil) at room temperature

of 25°C. After each measurement, in triplicate, the mean value was calculated for each group and experimental time interval.

## SOLUBILITY

The solubility of the materials was evaluated according to a previous study (9). Discs measuring 7x1mm (diameter x height) with materials remained at 37°C for 24h. During insertion of cements into the matrix, 5cm of nylon thread were included. The specimens were removed from the matrix and placed in a dehumidifier with silica. Stabilization of the mass of each specimen was checked in a precision balance (Adventurer-Ohaus, Model AR2140-Indústria de Balanças Ltda., São Bernardo do Campo, SP, Brazil). After determination of the initial mass (IM), each of the specimens was suspended by the nylon thread and placed inside a flask containing 10mL of distilled water. The flasks stored at 37°C for 7 days and were put into a dehumidifier to obtain the final mass (FM). The solubility (S) of the cements was obtained by calculating the mass loss of each sample expressed in percentage (3).

## ANTIMICROBIAL ACTIVITY ASSESSMENT: MODIFIED DIRECT CONTACT TEST (MDCT)

Roots of bovine teeth (mandibular incisors) were sectioned to obtain root dentin blocks measuring 5x5x0.7mm (width x length x thickness) and were sterilized in an autoclave at 121°C for 20min. Standard strains of *E. faecalis* (ATCC 29212) were used to prepare bacterial suspension in the concentration of  $1 \times 10^8$  UFC mL<sup>-1</sup>. The dentin blocks (substrate) were submitted to biofilm formation, and they were contaminated with 2mL of the inoculum in 24-well cell culture plates.

The plates were placed in a bench incubator with orbital agitation (model Q816M20, Quimis Aparelhos Científicos Ltda., Diadema, SP, Brazil) in a microaerophilic environment at 37°C for 14 days. The culture medium was completely changed every 48 hours.

The MDCT was performed according to the Vazquez-Garcia *et al.* (9). Cement discs (diameter=7mm, thickness=1mm) were fabricated. Six hours after manipulation, they were sterilized by ultraviolet radiation for 30 min, on each side, and placed on the dentin blocks containing the biofilm formed. For each experimental group, 6 discs (n=6) were used, as shown in Table 1. A control group (n=6) was included, in which the biofilm was placed in contact with Teflon discs. The time interval of contact was 15 h, and the discs were kept in an incubator at 37°C. After the contact period, the cement discs removed and the dentin blocks with remaining biofilm (including the control group) were individually placed in microtubes containing 1 mL of sterile saline solution and glass pearls (3.5±0.2 mm). The microtubes were agitated in a vortex (Model Q220, Quimis Aparelhos Científicos Ltda., Diadema, SP, Brazil) for 1 minute to detach and suspend microorganisms. Serial dilution was performed and three aliquots of 20µL of each of the dilutions were distributed in Petri dishes containing Tryptic Soy agar culture medium. The plates were then incubated at 37°C for 48 h for later counts of CFU mL<sup>-1</sup>.

The data obtained in the physicochemical tests were statistically analyzed by means of ANOVA and Tukey tests. The MDCT data were submitted to logarithmic transformation and statistically analyzed by means of the Kruskal-Wallis and Dunn tests, at a level of significance of 5%.

**Table 1.** Experimental groups and materials used.

Groups	Materials	Manufacturers
TSC/ZrO <sub>2</sub> +H <sub>2</sub> O	1gr. [70% Tricalcium silicate (TSC)*/30% ZrO <sub>2</sub> **] + 360μL distilled water	*Mineral Research Processing, Meyzieu, France **Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil.
TSC/ZrO <sub>2</sub> + 0.2%CTR	1gr.[70% TSC/30% ZrO <sub>2</sub> **] + 360μL distilled water 5,5mg/mL CTR**	**Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil.
TSC/ZrO <sub>2</sub> + 0.4%CTR	1gr.[70% TSC/30% ZrO <sub>2</sub> **] + 360μL distilled water 11mg/mL CTR**	**Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil.

## RESULTS

The results of the properties are presented in Table 2. TSC/ZrO<sub>2</sub>+CTR in the two concentrations showed higher IST values than those of TSC/ZrO<sub>2</sub>+H<sub>2</sub>O ( $P<0.05$ ). TSC/ZrO<sub>2</sub>+CTR 0.4% showed the lowest FST values ( $p<0.05$ ). After 1 day TSC/ZrO<sub>2</sub>+H<sub>2</sub>O demonstrated lower pH values than the associations with CTR ( $p<0.05$ ). In the other time

intervals, TSC/ZrO<sub>2</sub> and associations promoted similar alkalization ( $p>0.05$ ). TSC/ZrO<sub>2</sub>+CTR 0.4% presented lower gain in mass than the other materials ( $p<0.05$ ).

The results of the MDCT are represented in Table 3. Higher antibiofilm effectiveness was observed for TSC/ZrO<sub>2</sub>+CTR in the two concentrations in comparison with the positive control/C+ ( $p<0.05$ ).

**Table 2.** Mean and standard deviation of setting time (ST), pH and solubility of materials evaluated.

Groups		TSC/ZrO <sub>2</sub> +H <sub>2</sub> O	TSC/ZrO <sub>2</sub> + 0.2%CTR	TSC/ZrO <sub>2</sub> + 0.4%CTR	Control
ST (min)	IST	43.8 (±0.84) <sup>c</sup>	84.8 (±2.95) <sup>b</sup>	94.4 (±2.30) <sup>a</sup>	-
	FST	291.0 (±1.0) <sup>a</sup>	288.6 (±1.14) <sup>a</sup>	283.2 (±2.95) <sup>b</sup>	-
pH (days)	1	11.38 (±0.20) <sup>b</sup>	11.66 (±0.12) <sup>a</sup>	11.72 (±0.2) <sup>a</sup>	6.84 (±0.08) <sup>c</sup>
	3	10.32 (±0.35) <sup>a</sup>	10.09 (±0.68) <sup>a</sup>	10.36 (±0.36) <sup>a</sup>	6.77 (±0.16) <sup>b</sup>
	7	10.53 (±0.41) <sup>a</sup>	10.39 (±0.61) <sup>a</sup>	10.39 (±0.54) <sup>a</sup>	6.68 (±0.15) <sup>b</sup>
	14	10.50 (±0.30) <sup>a</sup>	10.37 (±0.52) <sup>a</sup>	10.65 (±0.22) <sup>a</sup>	6.39 (±0.13) <sup>b</sup>
	21	10.76. (± 0.29) <sup>a</sup>	10.61 (±0.44) <sup>a</sup>	10.49 (±0.45) <sup>a</sup>	6.67 (±0.16) <sup>b</sup>
Solubility (%-mass loss)*		-1.59 (±0.58) <sup>b</sup>	-1.63 (±0.39) <sup>b</sup>	-0.54 (±0.25) <sup>a</sup>	-

Different letters indicate statistically significant difference between experimental groups ( $p<0.05$ ). \*Negative values indicate mass gain. Control: distilled water.

**Table 3.** Mean and standard deviation of CFU mL<sup>-1</sup> log<sub>10</sub>

Groups	TSC/ZrO <sub>2</sub> +H <sub>2</sub> O	TSC/ZrO <sub>2</sub> + 0.2%CTR	TSC/ZrO <sub>2</sub> + 0.4%CTR	C+
CFU mL <sup>-1</sup>	4.52 (1.07) <sup>ab</sup>	1.37 (±2.15) <sup>b</sup>	0.0 (±0.0) <sup>b</sup>	8.04 (±0.27) <sup>a</sup>

Different letters indicate statistically significant difference between experimental groups ( $p<0.05$ )  
Positive Control - C+: Teflon disc.



## DISCUSSION

The null hypothesis was rejected since the addition of cetrimide (CTR) to the tricalcium silicate cement (TSC) associated with ZrO<sub>2</sub> interfered in some physicochemical properties; and increased the antibiofilm activity of the TSC. The aim of adding antimicrobial agents to materials is to increase their antimicrobial activity (25,26). Other substances have been mixed or associated to MTA to improve consistency and antimicrobial activity (8,9).

Cetrimide (CTR) has been used in association with irrigant solutions (22,23), as dentin pretreatment (24), and incorporated to intracanal medications (21) and root canal sealers (25-27). Giardino *et al.* (22) evaluated the antimicrobial, toxicity and cleaning effectiveness of ethylenediaminetetraacetic acid (EDTA) and maleic acid (MA) alone and combined with cetrimide (CTR), and showed that the addition of cetrimide to EDTA and MA removed accumulated hard-tissue debris effectively from the canal walls and increased their antimicrobial activity when compared to the same solutions without detergents. Another study performed the dentin pre-treatment by irrigating the root canal for 1 minute with 0.5% CTR, demonstrating its ability to reduce dentin surface tension (24).

The 0.2% CTR has shown antimicrobial activity against polymicrobial biofilm (23). Furthermore, 0.2% CTR improved the antimicrobial activity of experimental intracanal medications against *E. faecalis* (21). Therefore, the concentrations of 0.2 and 0.4% of CTR were used for this study. TSC/ZrO<sub>2</sub>+H<sub>2</sub>O and the cements modified with CTR showed setting time, pH and solubility in accordance with the specifications ISO-6876 standard, as observed for TSC-based materials (2,3,8). The setting time (ST) depends on the material components, particle size, temperature and relative humidity (27). A prolonged ST may favor the solubility and/or disintegration, or

displacement of the cement (28). Addition of CTR in the two concentrations increased the initial setting time (IST) of the TSC/ZrO<sub>2</sub> and reduced the final setting time (FST). CTR was associated as an aqueous solution in the same proportion as distilled water for TSC/ZrO<sub>2</sub> (1 g/360 µL). The FST values of the experimental cement modified with CTR were similar to TSC/ZrO<sub>2</sub>+H<sub>2</sub>O.

TSC have the ability to alkalize the medium (2). The alkaline pH is related to the antimicrobial effect (4). Antibacterial activity of calcium silicate-based endodontic sealers has been observed (29). All the experimental materials evaluated in this study showed the alkalinization ability. Our results are in agreement with those of other studies using tricalcium silicate repair materials (2,4,9,10).

Solubility of repair cement may reduce its sealing capacity, allowing bacterial and fluid infiltrations (30). The International standards determine solubility less than 3% (19,27,28). In the present study, the solubility test was performed in accordance with the modified methodology of Vasquez-Garcia *et al.* (9), in which test specimens were fabricated with standardized dimensions. The solubility values of all materials were negative, which means that they presented mass gain. The solubility values shown by the materials may be associated with the alkaline pH, the hydration process and porosity (3,4). The porosity of a material results in spaces between the unhydrated particles. Since these materials are considered hydrophilic, they capture water in the hydration process and can increase mass as the water is fixed in the structure. Thus, the increase in mass after 7 days in distilled water from TSC and associations probably occurred during the hydration process, increasing the mass of the materials. TSC and the associations showed an increase in mass, which can improve sealing properties. The incorporation of CTR into the AH Plus endodontic sealer reduced its solubility (20). The surfactant effect of CTR may

allow better cement hydration. Thus, the solubility of the association of TSC / ZrO<sub>2</sub>+0.4% CTR was lower than that of TSC without CTR.

Bovine root dentin contaminated with *E. faecalis* has been used to evaluate the antibiofilm effect of experimental materials (8), as in the present study. The MDCT showed that the addition of CTR in the two concentrations improved the antibiofilm activity of TSC/ZrO<sub>2</sub>. 0.2% cetrimide (CTR) promoted eradication of *Streptococcus mutans* biofilm in most specimens when applied to a biofilm dentin model (31). As a surfactant with antimicrobial properties which can weaken the cohesive forces of the biofilm, CTR disrupts the extracellular polysaccharide matrix (32). There are no studies about the association of CTR with TSC for comparison of the results. Calcium silicate-based sealers have antimicrobial activity due to the ability to release hydroxyl ions, increasing the pH (29). However, this activity against *E. faecalis* is limited. In the present study, the tricalcium silicate cement (TSC) associated with ZrO<sub>2</sub> increased the efficacy of the cement against *E. faecalis* biofilm. The association of CTR to AH Plus sealer also increased the antibiofilm activity, promoting the inhibition of *E. faecalis* biofilm (26).

In conclusion, CTR presented potential for promoting greater antibiofilm activity of the TSC cement, without harming its physicochemical properties of setting time, pH and solubility. Future studies must be conducted to evaluate other physicochemical and biological properties of this association.

#### DECLARATION OF CONFLICT OF INTEREST

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

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