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Ytterbium Oxide as Radiopacifier of Calcium Silicate-Based Cements. Physicochemical and Biological Properties

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This study evaluated physicochemical properties, cytotoxicity and bioactivity of MTA Angelus (MTA), calcium silicate-based cement (CSC) and CSC with 30% Ytterbium oxide (CSC/Yb_2O_3). Setting time was evaluated using Gilmore needles. Compressive strength was evaluated in a mechanical machine. Radiopacity was evaluated using radiographs of materials and an aluminum scale. Solubility was evaluated after immersion in water. Cell viability was evaluated by means of MTT assay and neutral red staining, and the mineralization activity by using alkaline phosphatase activity and Alizarin Red staining. The data were submitted to ANOVA, Tukey and Bonferroni tests (5% significance). The bioactive potential was evaluated by scanning electron microscopy. The materials presented similar setting time. MTA showed the lowest compressive strength. MTA and CSC/Yb_2O_3 presented similar radiopacity. CSC/Yb_2O_3 showed low solubility. Saos-2 cell viability tests showed no cytotoxic effect, except to 1:1 dilution in NR assay which had lower cell viability when compared to the control. ALP at 1 and 7 days was similar to the control. MTA and CSC had greater ALP activity at 3 days when compared to control. All the materials present higher mineralized nodules when compared with the control. SEM analysis showed structures suggesting the presence of calcium phosphate on the surface of materials demonstrating bioactivity. Ytterbium oxide proved to be a properly radiopacifying agent for calcium silicate-based cement since it did not affected the physicochemical and biological properties besides preserving the bioactive potential of this material.

Key Words: ytterbium, physicochemical analysis, silicate cement, endodontic, cell survival.

Introduction

Mineral Trioxide Aggregate (MTA) is a biomaterial composed of Portland cement (PC) (1) and bismuth oxide as radiopacifier (2). PC is calcium silicate-based cement (CSC) that contains other chemical compounds such as tricalcium aluminate and presents physicochemical, mechanical and biological properties similar to MTA (2,3). Calcium silicate cements might be suitable for biomedical applications as biomaterial for bone/dental repair (4). However, CSC do not have radiopacity to be distinguished from the surrounding anatomic structures (2,3).

Bismuth oxide (Bi_2O_3) reduces calcium hydroxide release, increases solubility, and causes deterioration in dimensional stability of MTA (5). Furthermore, lower cell viability was demonstrated for CSC with Bi_2O_3 in comparison with zirconium oxide and calcium tungstate (6). New radiopacifiers have been studied as alternatives to Bi_2O_3.

Ytterbium (Yb) is a very ductile metal that slowly reacts with water and are used in imaging exams (7). The incorporation of Ytterbium trifluoride (YbF_3) into glass ionomer cement promoted an increase in hardness of the material, and reduction in the setting and working times (8). The use of YbF_3 in the concentration of 30% was sufficient to promote proper radiopacity, without changing the properties of experimental cement (9). The association of PC with YbF_3 demonstrated radiopacity higher than 4 mm/Al and an increase in the compressive strength of the material (10).

The CSC-based cements are described as bioactive, stable materials, capable of promoting the hydroxyapatite crystal formation (11). Analysis of the structure and chemical composition of these materials has shown the formation of calcium carbonate and calcium hydroxide when immersed in distilled water, and the formation of calcium phosphate when immersed in PBS (11). The calcium ions released by MTA react with the phosphate of PBS, resulting in the formation of hydroxyapatite or carbonated apatite, components which are attributed to the mineralization ability of the calcium silicate-based cements (12,13).

Yb_2O_3 is a potential radiopacifier for calcium silicate-based cements. Therefore, the aim of this study are to confirm that the association of Yb_2O_3 with calcium silicate-based cement presents physicochemical and biological properties for use as dental repair material. The null

hypothesis is that there is no difference in physicochemical, biocompatibility and bioactivity of the calcium silicate-based cement (CSC), CSC with 30% Ytterbium oxide (CSC/Yb_2O_3), an experimental material, and the reference material (MTA) for endodontic purposes.

Material and Methods

The experimental materials and their powder-liquid ratios are in Table 1. CSC was evaluated in pure form or prepared in association with the 30% Yb_2O_3 (% by weight), which was mixed with distilled water using a powder/liquid ratio determined by previous tests to obtain consistency of retrofilling material and proper radiopacity.

The following tests were performed to evaluate the physicochemical and biological properties of the experimental materials.

Setting Time

The setting time was evaluated by dispensing the sealers into stainless steel molds measuring 10 mm in diameter and 1 mm high (n = 6). The materials were kept in an oven at 37 °C and 95% humidity during the experiment. The setting time test was performed in accordance with ISO 6876 (14). A Gilmore needle with a mass of 100 ± 0.5 g and 2 ± 0.1 mm in tip diameter was used. The setting time was determined as the mean time between beginning manipulation and the time elapsed until needle no longer produced marks on the cement surfaces.

Solubility

Circular molds were fabricated using polyvinyl siloxane impression material, based on the measurements recommended by Carvalho-Júnior et al. (15), with a thickness of 1.5 mm and internal diameter of 7.75 mm. Each mold was placed on a glass slide covered with cellophane paper film and filled with a material to be evaluated (n=6). A nylon thread was included in the mass of the cements. The set was stored in an oven at a temperature of 37°C and 95 % humidity for a period corresponding to three times the setting time of each material. The test specimens were removed from the molds, weighed on a precision balance HM-200 (A & D Engineering, Inc., Bradford, MA, USA) until stabilization of the mass (initial mass) and suspended by means of fixing the nylon threads inside plastic receptacles with lids, containing 7.5 mL of distilled and deionized water. The receptacles remained in the oven at 37°C for 7 days, when the test specimens were removed from the distilled water, dried and placed in a dehumidifying chamber, then to be weighed again until stabilization of the mass, thus obtaining the final mass. The loss of mass was expressed in percent of the original mass. Weighing procedures were repeated three times for each sample.

Table 1. Experimental materials, manufacturer and used ratios

Material	Proportion powder: liquid
Calcium Silicate-based Cement (CSC)*	Proportion: 1g powder: 330 µL distilled water
CSC + 30% Ytterbium Oxide** (CSC/Yb_2O_3)	Proportion: 1g powder: 330 µL distilled water
White MTA Angelus (MTA) ***	Proportion: 1g powder: 330 µL distilled water

*CSC - CPB-40 Estrutural Votoran, Votorantin Cimentos, Camargo Correa Cimentos S.A., Pedro Leopoldo, MG, Brazil). **Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil. ***MTA, Angelus, Londrina, PR, Brazil.

Compressive Strength

The cements were placed in a cylinder-shaped mold measuring 12 mm high and 6 mm in diameter. The set was placed in the oven. The set was stored at a temperature of 37°C and 95% humidity for a period corresponding to three times the setting time of each material. Then the samples were removed from the mold, and the flat surface of each sample was gently abraded with 600 grit abrasive paper. The samples remained in the oven (37 °C and 95% humidity) up to the time intervals of 24 h and 21 days, when they were submitted to the compression test in a universal testing machine (Emic DL Equipamentos e Sistemas de Ensaio, São José dos Pinhais, PR, Brazil) with a load cell of 5 KN and a speed of 0.5 mm/min. The maximum stress in MPa was calculated, using the maximum force and the diameter of the cylinders (1 MPa = 1N/1m^2).

Radiopacity

The radiopacity test was performed based on ISO 6876 (14) standards. Teflon rings with an internal diameter of 10 mm and height of 1 mm were used to fabricate 6 test specimens of each material. After manipulation, the materials were placed in the rings, which were kept at 37 °C and 100% humidity for 48 h until the materials set. After this time interval, a disc of each material was placed on an aluminum scale with occlusal film under it, and the samples were thus radiographed. Radiographs were taken with an X-ray appliance model GE 1000 (General Electric, Milwaukee, WI, USA), operating at 50 kV, 10 mA, 18 pulses per second, with a focal distance of 33 cm, on occlusal film (Insight - KodakComp, Rochester, NY, USA). The films were processed in an automatic developer (A/T 2000®XR, Ar Techniques Inc., Hicksville, NY, USA), digitized with a scanner, and evaluated using the UTHSCSA ImageTool for Windows version 3.00 software, in which the areas of each degree of the aluminum scale and the cements were selected, to determine the equivalence of radiopacity of the cements in millimeters of aluminum.

Cell Cultures and Preparation of Extracts of the Materials

The Saos-2 cells (ATCC HTB-85) were cultivated in T-75 flasks (Jetbiofil, Elgin, SR, USA), containing D-MEM medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, Life Technologies, Grand Island, NY, USA), penicillin (100 IU/mL) (Sigma-Aldrich), streptomycin (100 μg/mL) (Sigma-Aldrich) in 95% humidified atmosphere, 5% CO_2 and 37°C until confluence was reached.

To prepare the materials, one gram of powder was weighted and manipulated with distilled water, on a sterile glass plate. After preparation, the material was adapted to the bottom of a well of a 12-well plate and hydrated with dampened gauze. The plates were then maintained at 37 °C for 15 h to allow the complete setting reaction of the materials. After this time interval, the plates were exposed to ultraviolet light for 30 min, for disinfection, and 5 mL D-MEM culture medium, without FBS, was put into each well of the plates, and maintained at 37°C, 95% humidity and 5% CO_2 for 24 h. After 24 h, to observe the dose-response of each material, dilutions of the extracts (1:1, 1:2, 1:4 e 1:8) were made in D-MEM without PBS.

MTT Assay

Cell viability was determined by MTT assay. This assay is based on the capacity of the mitochondrial dehydrogenase enzyme to convert yellow tetrazolium 3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-tetrazolium (MTT; Sigma-Aldrich) into colored formazan compounds, whose absorbance is proportional to the quantity of live cells. After counting and plating the Saos-2 osteoblast cells ($1x10^5$ cells/mL) in 96-well plates with D-MEM culture medium with 10% FBS, the cells were exposed to the cement extracts (200 μL/ well) in dilutions of 1:1; 1:2; 1:4; 1:8 and control (cells not exposed to the extracts) for 24 h. After 24 h, the culture medium was replaced by 100 μL MTT (0.55 mg/mL) with incubation for 3 h. After this, each well was washed with 200 ul of phosphate buffer 1X (PBS 1X, Sigma-Aldrich), and 100 μL of isopropyl alcohol (HCl 0.04N isopropyl alcohol, Sigma-Aldrich) was added to each well to solubilize the formazan. The optical density (OD = 570 nm) was measured using an automated microplate reader (Elx800; Bio-Tek Instruments, Winooski, VT, USA). Three independent experiments were performed (n=12 per group).

Neutral Red (NR) Assay

Neutral red is a vital dye that is incorporated though endocytosis and accumulates in the lysosomes of cells that have intact membranes. For this assay, $1x10^5$ cells/mL were plated in 96-well plates with D-MEM medium with 10% FBS, which were incubated for 24 h. After this period, the cells were exposed to the cement extracts (200 μL/ well) for 24 h; the extracts were replaced with 100 μL of 50 μg NR / mL solution (Sigma-Aldrich), and then incubated at 37°C, 95% humidity and 5% CO_2 for 3 h. Afterwards, 100 μL of the solution (50% ethanol and 1% acetic acid) (Sigma-Aldrich) was used for solubilization. The optical densities were measured in a spectrophotometer (Elx800; Bio-Tek Instruments, Winooski, VT, USA) at a wavelength of 570 nm (16). Three independent experiments were performed (n=12 per group). According to the results of MTT and neutral red, the cement dilution selected for subsequent analyses was 1:8.

Alkaline Phosphatase Activity

Alkaline phosphatase activity was evaluated by means of the Labtest kit (Labtest, Lagoa Santa, MG, Brazil). The principle of the method is thymolphthalein monophosphate hydrolysis by alkaline phosphatase produced by the cells. After 24 h of plating ($1x10^5$ cells / mL in 96-well plates), Saos-2 cells were exposed to the cement extracts in the dilution of 1:8, throughout the time intervals of 1, 3 and 7 days. The eluted materials were renewed every two days throughout all of the 7 days of exposure of the samples. After each experimental time interval, all the cement extracts were removed and the cells were washed with 200 μL PBS 1X, followed by the addition of 200 μL of a lauryl sulfate solution (1% in distilled water, Sigma-Aldrich) to each well. The samples went through a period of rest for 30 min at room temperature. In accordance with the manufacturer's recommendation, 5 μL of each sample in lauryl sulphate solution was transferred to microtubes (Eppendorf, Hamburg, Germany), containing the enzyme substrate and buffer. The systems were incubated at 37°C for 10 min and then 500 μL of color reagent was added. The optical density was evaluated in an automatic microplate reader (ELx800, Bio-Tek Instruments, Winooski, VT, USA) at 590 nm. The assay was performed in sextuplicate and repeated three times in an independent manner (n=18/ group). The data were expressed as ALP activity normalized by the number of viable cells in the respective time interval of culture (OD = 570 nm).

Alizarin Red Staining (ARS)

The alizarin red stain is used to identify calcium deposits in cell cultures. In order to evaluate the calcium deposited by the cells exposed to the different calcium silicate-based materials, Saos-2 cells were counted and plated ($1x10^4$ cells/mL) in 12-well pates. For this experiment the culture medium was supplemented with 50 μg/mL of L-ascorbic acid (Sigma-Aldrich) and 10 mM β-glycerophosphate (Sigma-Aldrich). For 21 days, the D-MEM medium with and without the cement extracts (dilution 1:8) were renewed

every two days.

After this period the medium was aspirated, the wells were washed with PBS, and the cells fixed with 10% paraformaldehyde (Sigma-Aldrich) at room temperature for 15 min. The monolayers were then washed twice with distilled water, before adding 1 mL of alizarin red staining (2% - pH 4.1 from Sigma-Aldrich), and thus maintained at room temperature for 20 min. After this period, the dye was aspirated and the wells were washed 4 times with distilled water (1 mL). The plates were then slightly inclined to favor aspiration, and left for 2 min to facilitate excess water removal, and were immediately photographed (Canon EOS-1D, Canon Inc, Tokyo, Japan) and analyzed with the ImageTool program (Dental School of the University of Texas Health Science Center, San Antonio, TX, USA). Three independent experiments were performed (n=12 per group).

Bioactivity

The bioactivity test was performed based on Bosso-Martelo et al. (17). The materials were manipulated and adapted in cylindrical molds measuring 1 mm high X 7.5 mm in diameter, and remained in an oven at 95% humidity and 37 °C. After this period of setting, the samples were completely immersed in 5 mL a PBS solution (for 500 mL distilled water; anhydrous NaCl – 90 g; Na_2HPO_4 – 13.65 g; $Na_2H_2PO_2H_2O$ – 2.42 g). The test specimens remained immersed in PBS and kept in an incubator at 37 °C for 28 days. The PBS solution was not changed during the analysis period. After this period, silica gel and lime soda were placed in a receptacle, close to the samples, and maintained in the oven for 12 h to remove excess humidity from the materials, which were afterwards coated with carbon so that there would be electrical conductivity. Microstructural evaluation of the cement surfaces was performed by means of visualization in a scanning electron microscope SEM (JEOL, JSM-6610LV Scanning Electron Microscope, USA) in the secondary electron mode.

Statistical Analysis

Normality and equal variances tests were conducted prior to One-way ANOVA statistical analysis to guarantee that the data followed normal distribution and presented homoscedasticity. The physicochemical tests were submitted to Tukey´s comparison post-hoc test. Comparisons of cell viability and ALP activity were analyzed by two-way ANOVA with the Bonferroni post-test. Comparisons of alizarin red were performed by one-way ANOVA followed by the Tukey test. In all cases, the results were considered for a p value of less than 0.05.

Results

Table 2 presents the mean values of setting time, solubility, compressive strength and radiopacity tests.

Setting time of CSC/Yb_2O_3, pure CSC and MTA were similar (p>0.05). All the materials presented low solubility, and MTA and CSC/Yb_2O_3 were similar (p>0.05). The compressive strength of the association CSC/Yb_2O_3 and pure CSC were similar (p>0.05) (24 h and 21 days). Radiopacity of CSC/Yb_2O_3 and MTA was similar (p>0.05).

The cell viability using MTT assay showed that MTA, CSC and CSC/Yb_2O_3 presented no cytotoxic effect, since the cell viability was higher for MTA and CSC/Yb_2O_3 (p<0.05) in all dilutions (Fig. 1A). Therefore, the 1:8 dilution was selected for the bioactivity experiments. The NR assay complemented the results of the MTT assay, and also showed that MTA, CSC and CSC/Yb_2O_3 presented no cytotoxic effect, except for the dilution1:1, in which there was lower cell viability for of the materials in comparison with the control group. In the 1:8 dilution, cell proliferation was significantly higher for CSC/Yb_2O_3 (p<0.05), (Fig. 1B).

Figure 2 shows the structures that suggest the presence of calcium phosphate deposits on the surface of materials, demonstrating their bioactive potential.

The lowest cell viability and alkaline phosphatase activity were observed after one day of exposure (Fig. 3A and 3B). ALP activity increased after this time (Fig. 3B). On the third day of exposure, a higher ALP activity value was observed for the MTA and CSC groups when compared with control (p>0.05). On the seventh day of exposure, all the groups were similar to the control as regards alkaline phosphatase activity (p>0.05).

All materials induced a greater production of mineralized nodules when compared with the control (p<0.05). MTA and CSC produced more mineralized nodules than CSC/Yb_2O_3 (p<0.05) (Fig. 3C and 3D).

Table 2. Mean values and standard deviation of setting time, solubility, compressive strength and radiopacity tests

Tests	CSC	CSC/Yb_2O_3	MTA
Setting Time (min)	25.60^a (±1.34)	25.60^a (±2.60)	25.00^a (±2.82)
Solubility	1.14^b (±1.11)	0.05^a (±0.06)	0.03^a (±0.05)
Compressive Strength 24 h (MPa)	47.96^a (±7.45)	39.46^a (±5.78)	16.13^b (±3.95)
Compressive Strength 21days (MPa)	56.73^a (±19.83)	59.64^a (±14.60)	32.01^b (±7.76)
Radiopacity (mm/Al)	1.60^b (±0.27)	5.02^a (±0.43)	5.34^a (±0.50)

Values are mean ± standard deviation. Mean values followed by different superscript letter were significantly different (P<0.05) according to Tukey´s comparison post hoc test.

Discussion

The use of the CSC associated with new radiopacifiers has been studied as an alternative to MTA (17-19). A calcium silicate-based cement with ytterbium trifluoride (YbF_3) demonstrated interesting physical-chemical properties for use as biomaterial (10). The investigation of materials physicochemical properties is important to predict their clinical application (20). The incorporation of radiopacifiers to CSC may increase the material's setting time (10,18). Antonijevic et al. (10) observed a short setting time for the association of different calcium silicate-based cement with YbF_3 30% (6, 14 and 20 min). However, the present study using Yb_2O_3 showed that setting time was similar for CSC and CSC/Yb_2O_3. This result suggests that this radiopacifier does not interfere in the hydration process of the CSC.

All the materials presented solubility lower than 3%, in accordance with specifications ISO 6876 (14). Low solubility is a favorable property for the biomaterial, and may promote long-term bacterial sealing ability (21).

The presence of a radiopacifying agent is essential to enable differentiation of the material from the adjacent areas. The results of this study showed that except for pure CSC, the association of CSC/Yb_2O_3 and MTA had radiopacity higher than 3 mm/Al, in accordance with the recommendation of the ISO 6876 (14). In the present study, 30% of Yb2O3, by percentage of mass, was added to the CSC (CSC/Yb_2O_3), providing a material with proper radiopacity. This percentage is in agreement with Collares et al. (9) that associated 30% of the YbF3 in experimental dental cements.

The association of CSC/Yb_2O_3 did not change the mechanical strength of CSC, probably not changing the mechanism of hydration of CSC (18) and the compressive strength of these materials was higher than MTA. On the other hand, Prentice et al. (8) evaluated the addition of ytterbium fluoride (YbF3) to commercial glass-ionomer cement, and observed that the compressive strength decreased with the addition of YbF3. All the materials showed an increase in the compressive strength values after 21 days. According to Islam et al. (3) this occurred due to the continuous setting of the materials, which resulted in greater stability over the course of time.

The bioactive potential refers to the capacity of a determined material to be biocompatible and to stimulate the formation of mineralized tissue. These characteristics are observed in MTA and materials based on calcium silicate (22). The bioactive potential of MTA is attributed to the capacity for mineralization of PC components (12). Hydration of PC results in the deposition of calcium hydroxide on the surface of the material, which produces hydroxyapatite in the presence of phosphate-containing fluids (13). In the

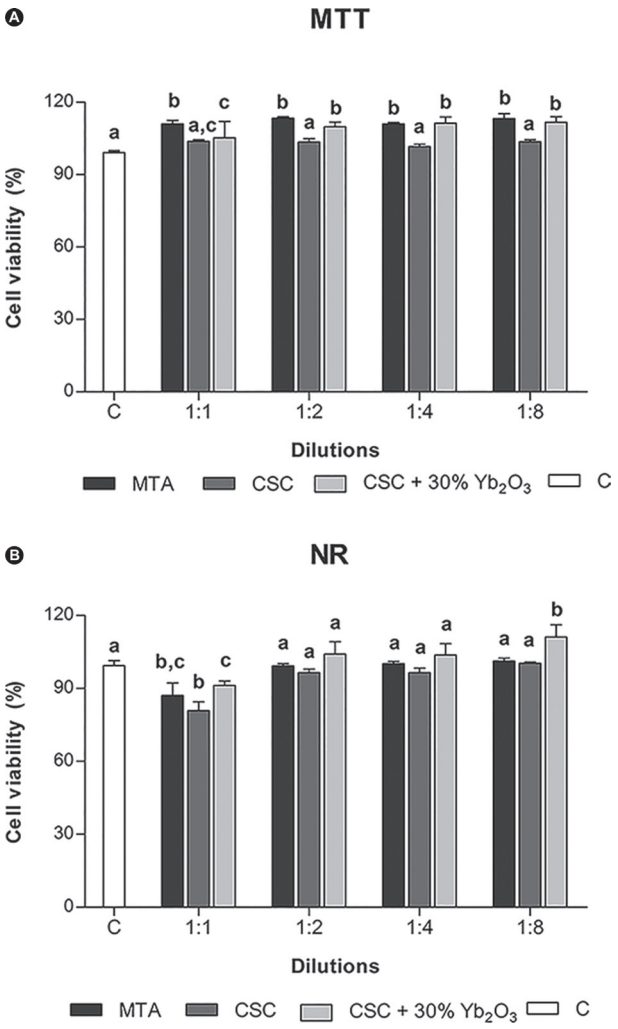

Figure 1. Viability of human osteoblast cells (Saos-2) evaluated by MTT (A) and NR (B) assays after 24 h of exposure to different dilutions of the cements (1:1, 1:2; 1:4 and 1:8) CSC; CSC/Yb_2O_3 and MTA, to culture medium without FBS (control). Bars with different letters represent statistically significant difference between extracts of the cements and control group. ANOVA and Bonferroni ($p<0.05$). CSC = calcium silicate-based cement; calcium silicate-based cement associated with 30% ytterbium oxide radiopacifier; and MTA = Mineral Trioxide Aggregate; and C = control.

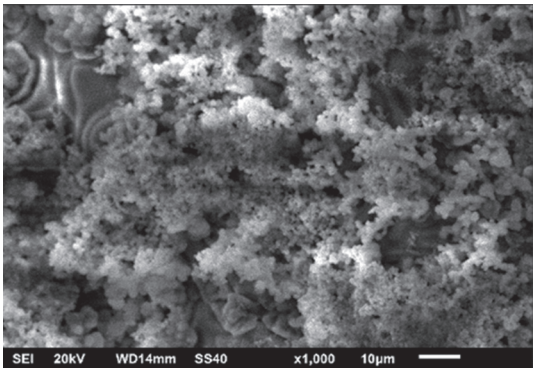

Figure 2. Spherical structures, suggesting bioactivity of the material (calcium phosphate).

present study, structures were observed in the SEM analysis suggesting the presence of calcium phosphate deposits on the surface of all the materials. These results suggested the biological potential of CSC and its association CSC/Yb_2O_3.

Another essential property of a biomaterial is the biocompatibility allowing the tissue repair. Calcium silicate-based cements have demonstrated potential for use as reparative materials and the interaction of the reparative materials with osteogenic cells is important to repair processes (22). In the 1:8 dilution, in comparison with the control group, higher cell viability was observed for CSC/Yb_2O_3. MTT assay showed that the MTA, CSC and CSC/Yb_2O_3 cements have no cytotoxic effect on human osteoblast SAOS-2 cell lineage. Antonijevic et al. (10) evaluated the biocompatibility of bioactive endodontic cements on mesenchymal cell differentiation, and showed that materials with YbF3 did not change the integrity of cells from bones of rats.

ALP is one of the main enzymes expressed during the early maturation of osteoblasts, and plays an important role in mineral deposition. Although MTA and CSC had showed grater ALP activity after 3 days in comparison to CSC/Yb_2O_3), the addition of ytterbium oxide as radiopacifier did not affected ALP activity as it was similarly expressed by Saos-2 cells in comparison to the control in all evaluated periods. It has been reported in other studies that the addition of radiopacifying agents did not affect the ALP activity and also allow the anatomical structures differentiation (22, 23). The evaluation of this enzyme allows the determination of the material bioactivity and its potential to promote repair with formation of mineralized tissue (20). The capacity of MTA to induce calcium deposition, as well as its bioactivity has been demonstrated (23). MTA Angelus cement stimulated the formation of mineralized tissue by the formation of

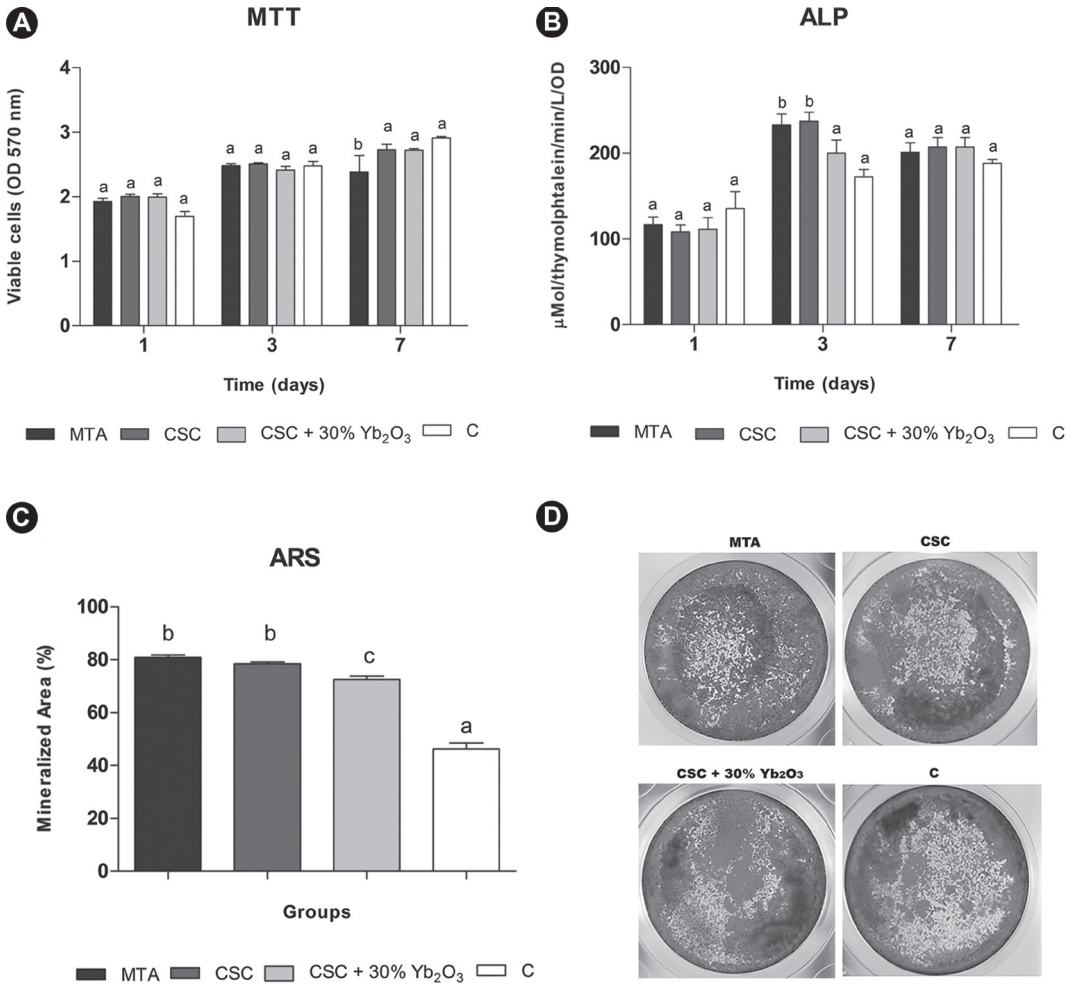

Figure 3. (A) Cell viability of cell lineage Saos-2; and (B) Alkaline Phosphatase Activity (ALP) evaluated after exposure to extracts of the cements CSC; CSC/Yb_2O_3 and MTA in the dilution of 1:8, and culture medium (control) in time intervals of 1, 3 and 7 days. (C) Alizarin red staining (ARS). (D) Images of mineralized area with 2% Alizarin red staining. Bars with different letters represent significant differences between cement extracts and negative control group in each period. CSC = calcium silicate-based cement; calcium silicate-based cement associated with 30% ytterbium oxide radiopacifier; and MTA = Mineral Trioxide Aggregate; and C = control.

calcium carbonate (24).

The alizarin red is a stain that identifies calcium deposits in cell cultures (22). The formation of calcified nodules has been related to the differentiation of stem cells into cells capable of forming mineralized tissues (25). In the present study, all the materials produced mineralized nodules after 21 days of cell cultures in an osteogenic medium (Fig. 3D).

Based on the result, Yb_2O_3 may be considered a potential radiopacifying agent for use in association with CSC.

Resumo

Este estudo avaliou as propriedades físico-químicas, citotoxicidade e bioatividade do MTA Angelus (MTA), cimento à base de silicato de cálcio (CSC) e CSC com 30% de óxido de itérbio (CSC/Yb_2O_3). O tempo de presa foi avaliado usando agulhas Gilmore. A resistência à compressão foi avaliada em uma máquina mecânica. A radiopacidade foi avaliada utilizando radiografias dos materiais e uma escala de alumínio. A solubilidade foi avaliada após imersão em água. A viabilidade celular foi avaliada por meio do ensaio MTT e coloração de vermelho neutro (NR), e a atividade de mineralização por meio da atividade da fosfatase alcalina e a coloração com Vermelho de Alizarina. Os dados foram submetidos aos testes ANOVA, Tukey e Bonferroni (5% de significância). O potencial bioativo foi avaliado por microscopia eletrônica de varredura. Os materiais apresentaram tempo de presa semelhante. O MTA mostrou menor resistência à compressão. MTA e CSC/Yb_2O_3 apresentaram radiopacidade semelhante. CSC/Yb_2O_3 apresentou menores valores de solubilidade. A viabilidade celular realizada pelos ensaios de MTT e NR não revelaram efeitos ctotóxicos em todas as diluições, exceto na diluição 1:1 no NR, o qual mostrou baixa viabilidade celular ($p<0.05$) em todos materiais testados quando comparado ao controle. A atividade de ALP em 1 e 7 dias foi similar ao controle ($p>0.05$). MTA e CSC tiveram significante aumento na atividade de ALP aos 3 dias quando comarados ao controle ($p>0.05$). Todos os materiais apresentaram grande produção de nódulos mineralizados quando comparados ao controle ($P<0.05$). A análise da SEM mostrou estruturas que sugerem a presença de depósitos de fosfato de cálcio na superfície dos materiais demonstrando bioatividade. O Yb_2O_3 mostrou ser um agente radiopacificador adequado em cimentos à base de silicato de cálcio uma vez que não afetou as propriedades físico-químicas e biológicas e ainda preservou o potencial bioativo desse material.

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