A New Calcium Silicate–Based Root Canal Dressing: Physical and Chemical Properties, Cytotoxicity and Dentinal Tubule Penetration

Natália Villa[1], Vanessa Valgas Dos Santos[2], Ubirajara Maciel da Costa[3], Aline Teixeira Mendes[1], Pedro Henrique Marks Duarte[1], Ricardo Abreu da Rosa[1], Jefferson Ricardo Pereira[4], Marcus Vinícius Reis Só[1]

[1]Conservative Dentistry Department, School of Dentistry, UFRGS - Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[2]Department of Post-Graduation in Environment and Health, UNIPLAC - Universidade do Planalto Catarinense, Lages, SC, Brazil
[3]Department of Veterinary Medicine, UDESC - Universidade do Estado de Santa Catarina, Lages, SC, Brazil
[4]Department of Prosthodontics, Unisul - Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil

Correspondence: Marcus Vinícius Reis Só, Rua Ramiro Barcelos, 2492, 90035-003 Porto Alegre, RS, Brasil. Tel: +55-51-9-9967-8504. e-mail: endo-so@hotmail.com

The aims of this study were to evaluate the physical and chemical properties, cytotoxicity and dentinal tubule penetration of a new calcium silicate-based root canal dressing. For pH and calcium ion release evaluation (1, 24, 72 and 168 h) were used a pH meter and colorimetric spectrophotometer, respectively. Radiopacity evaluation followed the ISO 6876:2012. Cytotoxicity was evaluated by the percentage of cell viability using MTT assay. Illustrative images of dentinal tubule penetration were obtained using confocal laser scanning microscopy (CLSM). Data from pH and calcium ion release were statistically analyzed by two-way analysis of variance and Tukey test. Radiopacity was analyzed using the Student t-test. The statistical tests for cytotoxicity results were the one-way analysis of variance and Tukey test. Both materials showed alkaline pH in all experimental times. The pH values for calcium hydroxide paste were higher than bioceramic paste at 1, 24, and 72 h ($p<0.05$). The calcium ion release of bioceramic was lower than the calcium hydroxide paste only at 24 h ($p<0.05$). The bioceramic was more radiopaque than the calcium hydroxide paste ($p<0.05$). Bioceramic paste presented a dose and time-dependent cytotoxic effect after MTT assay. CLSM images showed absence of tubule penetration for both pastes. The new calcium silicate-based canal dressing presented alkaline pH, high calcium release, and acceptable radiopacity. Bio C Temp showed a dose and time-dependent cytotoxic and absence of dentinal tubule penetration.

Introduction

The presence of microorganisms in the root canal system is directly related to the setting up of apical periodontitis (1). The chemical and mechanical preparation and root canal filling promote the reduction of this microbiota (2). However, the presence of anatomical complexities may limit the access of instruments and irrigation solutions, which favors the permanence of microorganisms organized in biofilms (3). The employment of intracanal medication helps to optimize disinfection, supplementing the previously mentioned procedures (4).

Calcium hydroxide is the most commonly used medication. It has an alkaline pH (approximately 12.8), low solubility, and adequate flow (5). In an aqueous solution, this medication dissociates into calcium and hydroxyl ions; this mechanism is responsible for several biological properties, which are attributed to this medication, such as antimicrobial action, induction of hard tissue deposition and inhibition of tooth reabsorption (5). An adequate radiopacity is essential for verifying the correct placement and maintenance of the root canal dressing into the root canal and is achieved by the addition of substances such as zinc oxide, iodoform, and barium sulfate (6).

The bioceramic materials are designated for repair and reconstruction in the medical and dental areas (7). They are widely recognized in Endodontics because they are employed as calcium silicate cement in procedures of root canal filling, apexification, revascularization therapy, root-end filling, conservative pulp treatments, perforations, and resorptions (8). They have excellent biocompatibility and can form a chemical bond with the dental structure and to be employed in humid environments (9). Besides, they promote excellent sealing and present adequate radiopacity (9).

The growing interest regarding these materials is evidenced by the development of new bioactive calcium silicate-based medications. Bio-C Temp (Angelus Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil) was launched on the market in 2020 as an alternative to calcium hydroxide in pulp necrosis, retreatment, persistent lesions or exudate and when sinus tract is present as described by the manufacturer. The chemical composition of Bio-C Temp includes glycol salicylate, titanium oxide, calcium tungsten, calcium aluminate, calcium oxide, and calcium silicate.

This study aimed to evaluate pH, calcium ion release, radiopacity, and dentinal tubule penetration of a new

calcium silicate-based intracanal dressing (Bio-C Temp) and a calcium hydroxide-based material (Ultracal XS; UltraDent Product, Inc., Indaiatuba, SP, Brazil), as well as verify the Bio-C Temp cytotoxicity. The null hypothesis was that the materials tested would present similar properties at the evaluations.

Material and Methods

The study was approved by the Ethics Committee of the Federal University of Rio Grande do Sul (CAEE-03668018.1.0000.5347).

A calcium silicate-based intracanal dressing (Bio-C Temp) and a calcium hydroxide-based intracanal dressing (Ultracal XS) were tested and compared regarding some physicochemical properties. The calcium hydroxide-based material was used as the control group. Both pastes were presented in a pre-filled multidose syringe with specific tips. An analytical balance (BEL Engineering; Milano, Italy) was employed to weigh each paste according to the test to be performed.

pH

In order to determine the pH and calcium ions release of the studied intracanal dressings, five specimens for each experimental time of each group (n=5) were produced at polyethylene tubes (length of 10 mm and an internal diameter of 1.0 mm) with a closed-end (10). An analytical balance (Adventurer, Ohaus Corp., USA) was used to weigh 0.206 g of each paste. The pastes were inserted into the tubes using 1 mL syringes up to the complete fulfillment. Each specimen was placed in a flask containing 10 mL deionized water and stored at 37 °C. The pH assessment was performed after 1, 24, 72, and 168 h of immersion. Previous to the readings, the paste samples were removed from the flasks, and the solutions were manually agitated for 5 s. The pH was measured with a digital pH meter (Digimed DM-22, São Paulo, SP, Brazil) previously calibrated with pH 7.0 and 4.0 buffer solutions (Synth Ltda, Diadema, SP, Brazil).

The control for this method was performed by a reading of the pH values of deionized water in which no samples were immersed.

Calcium Ion Release

The evaluation of calcium ion release was performed at the same experimental periods used for pH analysis (1, 24, 72, and 168 h). The calcium levels contained in the collected samples were analyzed with a colorimetric method employing the Arsenazo III reagent.

Standard Curve: One set of standard stock was used and prepared from Orion Calcium Standard, contains 15, 30, 45, 60 and 75 ppm calcium. For determination of calcium 100 µl of each standard is added to 5 ml of diluted calcium

reagent in a 10-ml plastic test tube and mixed thoroughly. The solution is read against a reagent blank at 650 nm. The calcium reagent develops immediately.

Samples: Aliquots of 15 µL obtained of each sample were used for calcium analysis. The sample volumes are added to the calcium reagent, mixed and read as described for the standards.

Radiopacity

Following the International Standard Organization (ISO) 6876:2012 recommendation, three specimens (n=3) were produced for each group. The medications were introduced into a matrix of 10 mm of diameter and 1 mm of height for sample production. Following that, the samples were laterally positioned on both sides of a digital sensor (RVG 5200, Carestream Health, Rochester, NY, USA) close to an aluminum scale, which was centrally placed. This scale thickness ranged from 0.5 mm to 5 mm. The next step was the radiography procedures using a Timex 70E X-ray device (Saevo, Ribeirão Preto, SP, Brazil) with an exposure time of 0.1 s. Images were analyzed with ImageJ software (Research Services Branch, National Institutes of Mental Health, Bethesda, MD, USA). The gray levels (pixel densities) of the aluminum scale and a 1.5 mm² standardized area in the center of the samples were calculated regarding their mean values and standard deviations. The radiopacity value was determined according to the radiographic density, which was also converted into millimeters of aluminum (mm Al).

Cytotoxicity

VERO Cell lines, from American Type Culture Collection (ATCC), were maintained in DMEM (Dulbecco's Modified Eagle Medium, Sigma-Aldrich, Darmstadt, Germany) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics (1% penicillin-streptomycin-gentamycin; Invitrogen) in 75-cm² culture flasks in a humidified atmosphere at 37°C and 5% CO_2.

Extract Preparation

The extract was prepared as recommended by ISO 10993-5, where 100 mg of Bio-C Temp was added to 1mL of DMEM medium with 5% of fetal bovine serum. The material was vortexed until completely suspended, then incubated for 24 h at 37°C. The solution was filtered in a 0.22 µL M, and a serial dilution was performed in DMEM with serum for final concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL and 1.5625 mg/mL.

Cytotoxicity Measurements by the MTT Technique

The cytotoxicity of Bio-C Temp was evaluated by the percentage of cell viability, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliu bromide (MTT) assay. The cells

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1×10^5 cells/well were seeded in a 96-well plate with 100 μL DMEM complete medium. After 24 h of incubation, the supernatant was replaced by different concentrations of Bio-C Temp extract (100 μL) and incubated in standard culture conditions for 8 or 24 h. Negative control cells were incubated only with DMEM complete medium. After this period, the medium was removed, and 50 μL MTT (5 mg/mL in DMEM) was added to each well and incubated for 2 h at 37 °C. Then, 100 μL dimethyl sulfoxide (DMSO) was added to each well to dissolve formazan crystals. The optical absorption was read at 490 nm wavelength, and the graph confection was performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, USA). All experiments were conducted in triplicate.

For cytotoxicity evaluation the parameters described by Dahl et al. (11) were applied in which cell viability was compared with control, and the result was defined in different categories as non-cytotoxic (>90% cell viability); slightly cytotoxic (60–90%); moderately cytotoxic (30–59%); and strongly cytotoxic (<30%).

Dentinal Tubule Penetration

The dentinal tubule penetration was evaluated through confocal laser scanning microscopy (CLSM). Fourteen mandibular human incisors were selected. The access cavities were performed with #1012 diamond burs, the working length was established, and root canal preparation was carried out with WaveOne Gold # 35.06 reciprocating system. During the preparation, the root canal was irrigated with 5 mL of 2.5% sodium hypochlorite using a syringe with a 30-diameter needle (Navitip; Ultradent Products Inc.). After that, the final irrigation protocol was done: the root canal was irrigated with 5 mL of 2.5% sodium hypochlorite using a syringe with a 30 diameter needle (Navitip; Ultradent Products Inc.); next, the root canal was rinsed with 5 mL of 0.9% saline solution and 2 mL of 17% EDTA (Maquira, Maringá, PR, Brazil). The EDTA was stirred for 3 min with a 20 K-file (Dentsply-Sirona, Ballaigues, Switzerland). A final rinse with 2 mL of 0.9% saline solution was performed one more time. The canals were dried with WaveOne #35.06 sterile paper points, and

the access cavities were then filled with CAVIT (3M ESPE, Seefeld, Germany). The roots were randomly distributed in two groups: Ultracal XS (n=10) and Bio-C Temp (n=10). Fluorescent calcium indicator (Fluo 3 - Thermo Fisher Scientific, USA) was added to the intracanal dressings in order to provide fluorescence in the CLSM. The Ultracal XS and Bio-C Temp were inserted into the canals with a Lentulo spiral. Finally, one week later, the specimens were transversally sectioned at 2 mm, 7 mm, and 12 mm from the root apex. Samples were examined from coronal to the apex using an Olympus Fluoview 1000 scanning confocal microscope (Olympus Corporation, Tokyo, Japan). Dentin impregnated area was analyzed at x10. The images recorded were 70 μm depth, with 800 x 800 pixels. Each image was analyzed by using ImageTool 3.0 software (UTHSCSA, San Antonio, Texas, USA).

Statistical Analysis

Data from the pH, calcium release, radiopacity, and MTT assay were analyzed using SPSS version 22.0 statistical software (SPSS, Inc., Chicago, IL, USA). The pH and calcium ion release values along the experimental times were statistically compared using two-way analysis of variance and Tukey post hoc test. The pairwise comparison of pH and calcium ion release values between both pastes in each experimental time was performed using the Student-T test. Radiopacity was analyzed using the Student-T test. One-way analysis of variance and Tukey's multiple comparison test compared the cytotoxicity results. The significance level was set at 5%. The dentinal tubule penetration had a descriptive analysis.

Results

Table 1 presents the means and standard deviation of the pH values. The pH values for calcium silicate-based material increased over the experimental period, with differences with the calcium hydroxide paste at the period of 1, 24, and 72 h ($p< 0.05$). In the intra-group analysis, the Bio-C Temp showed similar pH values only at 72 and 168 h, while the Ultracal paste presented a difference at 72 h when compared to the other experimental periods ($p<0.05$).

The Bio-C Temp presented an increasing calcium release pattern, and, at 1 h, a significant difference had occurred when compared to the other experimental periods ($p<0.05$). At the intergroup analysis, the calcium release of bioceramic material was lower than the calcium hydroxide

Table 1. Means and standard deviation of the pH values and calcium ion release (mg/L)

	1 h	24 h	72 h	168 h
Ultracal XS pH	10.23 [Aa] ± 0.27	10.69 [ABa] ± 0.18	11.60 [Ca] ± 0.1	11.01 [Ba] ± 0.50
Ultracal XS Ca+	221.97 [Aa] ± 73.52	314.09 [Ba] ± 2.72	308.33 [Ba] ± 6.83	313.89 [Ba] ± 2.46
Bio-C Temp pH	8.57 [Ab] ± 0.60	9.66 [Bb] ± 0.32	10.69 [Cb] ± 0.16	10.79 [Ca] ± 0.11
Bio-C Temp Ca+	152.08 [Aa] ± 52.11	298.40 [Bb] ± 11.56	312.94 [Ba] ± 5.57	313.89 [Ba] ± 4.81

Same capital letters in the row do not differ statistically by two-way ANOVA and Tukey's post-hoc. Same lowercase letters in the column do not differ statistically by Student's t Test. Significance level of 5%.

paste only in 24 h (p<0.05). Table 1 presents the values of calcium ion release. Additionally, the bioceramic material was significantly more radiopaque than the calcium hydroxide paste (p<0.05) (Table 2).

The MTT assay results showed that Bio-C-Temp presented a cytotoxic effect in a dose and time-dependent manner (Bar chart A and B). After 8 h of treatment, the doses of 100 mg/mL (51.85±3.80) and 50 mg/mL (42.27±0.67) presented a moderate cytotoxicity, and the doses of 25 mg/

mL (68.58±3.04) and 12.5 mg/mL (79.44±2.70) a slightly cytotoxic effect (p< 0.05). Moreover, cells treated with Bio-C-Temp after 24 h of incubation presented a strong cytotoxic effect at the doses of 100 mg/mL (14.58 ± 0.35), 50 mg/mL (10.49±0.30), and 25 mg/mL (19.42±0.78) (p<0.05). In contrast, the concentration of 12.5 mg/mL (60.66±0.51), 6.5 mg/mL (70.52±0.12), 3.12 mg/mL (83.87±2.01) demonstrated a slight cytotoxic effect (p<0.05) (Fig. 1).

Figure 2 shows CLSM images of bioceramic (A, B, and C) and calcium hydroxide (D, E, and F) paste with absence of tubule penetration.

Discussion

The materials tested in this study represent two types of root canal dressing with different bases, namely Bio-C Temp and Ultracal XS. Calcium hydroxide, the main base of Ultracal XS, was included in this study as a control group

Table 2. Means and standard deviation of the radiopacity (mm/Al)

Radiopacity	Means and standard deviation
Ultracal XS	5.1233^A ± 0.5408
Bio-C Temp	7.1500^B ± 0.7502

Different capital letters in the column are statistically different at the Student's T-Test. Significance level of 5%.
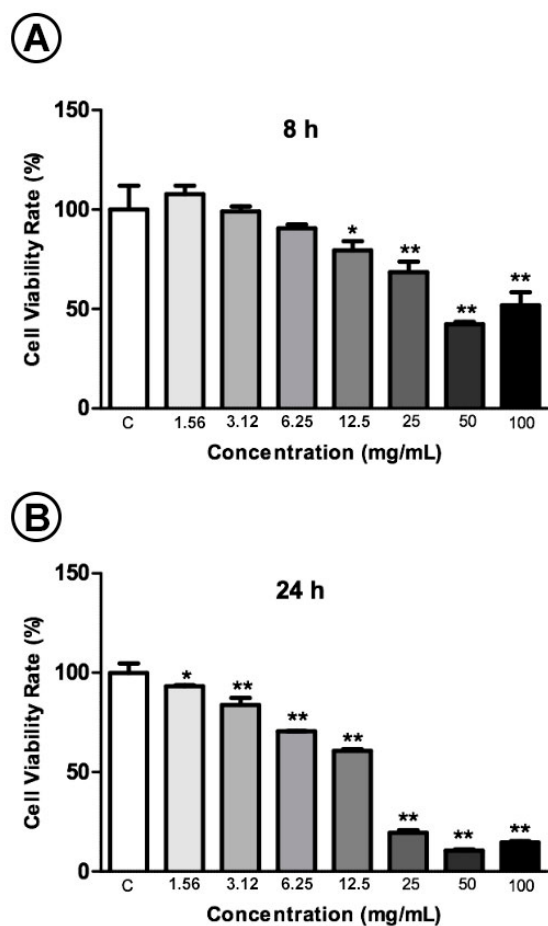

Figure 1. A and B: The viability of VERO cells exposed to 8 and 24-h extracts of Bio-C Temp. Cytotoxicity was determined by the MTT assay, and the data represent the average percentage of cell viability in three independent experiments conducted in triplicate. (**p<0.0001 and *p<0.01).
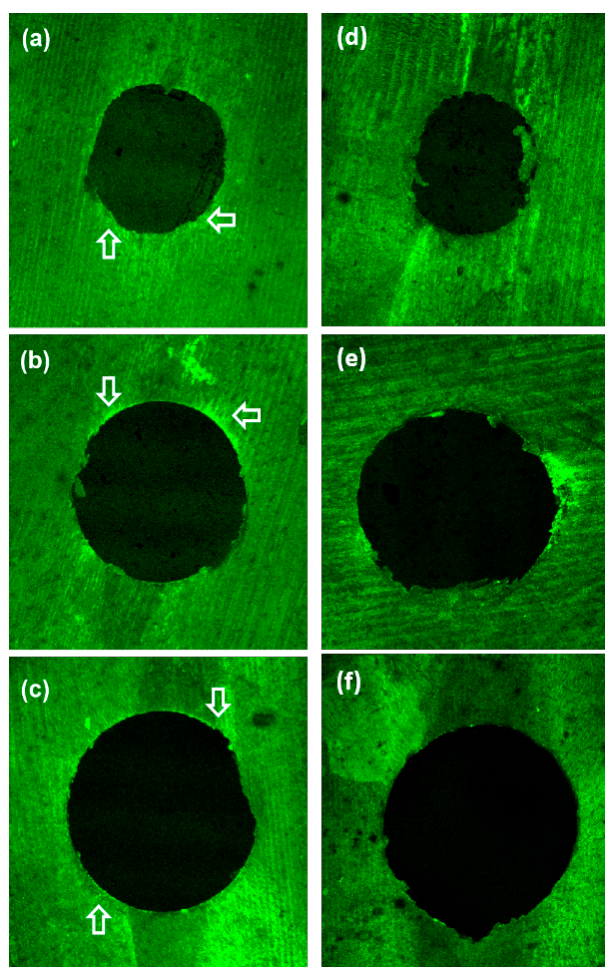

Figure 2. (a), (b), and (c) CLSM images showed absence of tubule penetration of Bio-C Temp group in the apical, middle and cervical third, respectively. The arrows indicate the presence of bioceramic past only at the periphery of the root canal. (d), (e), and (f) CLSM images also showed calcium hydroxide group with absence of tubule penetration in the apical, middle, and cervical canal third, respectively

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because it was considered the most commonly employed medication to complement the chemical-mechanical preparation of the root canal. The bioceramic materials were developed to set and harden into a cohesive whole in the presence of moisture. On the other hand, Bio-C Temp, which also contains bioceramic particles, is a temporary material intended to be removed after a while. In this sense, according to the manufacturer, the calcium silicate particles will hydrate in the presence of water, forming the hydrated calcium silicate on the particle surface. However, polymeric chains present in the product will hinder the connectivity of the hydrated particles, thus restricting the hardening of the material.

The clinical implication for non-hardening is that this material behaves like a paste, be used temporarily, and can be removed from the root canal using protocols similar to those used for calcium hydroxide paste. We believe that the usage time can be the same used for calcium hydroxide pastes, however other studies need to be carried out for this to be proven.

This material contains tricalcium silicate, dicalcium silicate, and calcium oxide. These components, when hydrated in the oral environment, form calcium hydroxide. Calcium hydroxide formation occurs, and rapidly it dissociates into Ca^{++} and OH^- ions increasing the pH of the medium. Nevertheless, the low solubility of calcium hydroxide has clinical importance, as it is responsible for the slow release of OH^- ions and the very low resorption of the material.

The released hydroxyl ions have a significant role in the action mechanism of calcium hydroxide, as they are responsible for a significant increase in the pH levels in the surrounding tissue and therefore acting as an anti-bacterial against bacteria proliferation. Furthermore, this calcium hydroxide, when dissociated, will react with the phosphate ions from the dentin, and they form a mineral zone infiltration (12).

The tested hypothesis was rejected since significant differences were observed for pH, calcium release, and radiopacity between the medications studied. Bio-C Temp presented pH values that were increasing over the experimental period, differing from calcium hydroxide with lower values at the periods of 1, 24, and 72 h (p<0.05). The pH and calcium release results for Ultracal XS in this study were similar to the findings of other research (13). Ultracal showed the highest pH and calcium release values. pH values for the Ultracal were always above 10 (13).

At all experimental periods, both medications were alkaline. The alkaline pH may favor the elimination of several microbial specimens commonly found in infected root canals (4) It is also suggested that the alkaline pH, together with the calcium ion release capacity, promotes

the mechanism of repair stimulation by deposition of mineralized tissue (14).

The calcium silicate-based paste presented an increasing release of calcium hydroxide ion at all experimental periods and exhibited a significant difference at 1 h (p<0.05). When compared with the control group, calcium silicate-based paste demonstrated a lower release pattern at 24 h (p<0.05). It may be inferred that the calcium release of the materials is essential for their microbiological and biological properties (13). The calcium reacts with the carbon dioxide, reducing the respiration source of anaerobic bacteria (14). According to Parirokh and Torabinejad (15), the presence of calcium may favor alkaline pH, providing a biochemical effect that is responsible for accelerating the healing process. The results of the calcium release of Ultracal XS paste are in line with the study by Só et al. (13), who showed high values at all experimental times for this material. The release of calcium ions occurred gradually in the Ultracal XS paste.

The colorimetric method employing the Arsenazo III is an analytical procedure that may be used to analyze many types of samples of interest to dental research. It has distinct advance in that: relatively simple and fast; require no elaborate equipment (spectrophotometry rather than atomic absorption or fluorescence); calcium can be analyzed by reagents so sensitive that, in most cases, microliter sample volumes are used, allowing the analysis to be repeated if desired (16).

Radiopacity is an essential feature for intracanal dressings because it allows the visualization of the root canal filling and the paste solubilization over time. As there is no ISO standard to verify the intracanal medication radiopacity, the protocol of the tests was based on ISO 6876:2012 for the present study, designed for root canal filling materials. In this research, the calcium silicate-based paste showed to be significantly more radiopaque than the calcium hydroxide paste, with values equivalent to 7.15 mm Al and 5.12 mm Al, respectively. The difference can be explained by the calcium tungstate and titanium oxide used as radiopacifier in Bio-C Temp.

Different methods have been proposed to estimate the in vitro cytotoxicity of endodontic materials. In the present study, the reduction of MTT by active cells showed a cytotoxic effect of Bio-C Temp. This toxicity could be a response to the high alkalinity since the calcium ion produces a positive effect on cell proliferation (17). The calcium ions release and the alkalization of the medium contribute to antibacterial properties (18).

MTT assay has been considered as the 'gold standard' method for determination of cell viability (19-21). However, the conversion to formazan crystals depends on metabolic rate and number of mitochondria, which is associated to:

acidic pH, polyphenols, pyruvate analog, and nanomaterials. (19-21). According to the MTT results, Bio-C Temp presented higher cytotoxicity at higher concentrations and slight cytotoxicity at lower concentrations in both periods (8 and 24 h). Therefore, the Bio-C Temp cell viability was dose time-dependent. The results of this study cannot be compared with other studies, but the possible biologic activity of bioceramic root canal dressing could be associated with their alkaline pH, a more significant release of Ca++.

Fluo 3 is a non-fluorescent compound, even though fluorescence significantly increases after binding to calcium (22). The calcium present in the calcium silicate intracanal dressing binds to Fluo 3 and, consequently, the fluorescence observed at microscopy belongs to bioceramic material. The present study investigated the penetrability of Bio-C Temp and the Ultracal XS inside dentinal tubule from human mandibular incisors. To our knowledge, only one study (23) has used Fluo 3 associated with calcium silicate-based sealers and posterior assessment at CLSM. Therefore, Fluo 3 may be the fluorophore of choice when the chemical element of calcium is present. CLSM produces light at a specific wavelength (488-600nm); the fluorophore is capable of increasing its fluorescence up to 6 times, depending on calcium quantity (22).

Furthermore, it was initially created to detect gradients of intracellular calcium ions. Through CLSM and for flow cytometry in biochemistry (22). It is worth emphasizing that Fluo 3 is not able to detect calcium ions from dental structure; therefore, the obtained results consist only from calcium on bioceramic material composition (23). In this study, it was not observed dentinal tubule penetration of the calcium silicate-based intracanal dressing and the Ultracal XS, only its presence at the periphery of the root canal (arrows in Fig. 2).

Bioceramic materials create a mineral apatite interface between the dentinal wall and the material (12), but it was not studied yet for this material. The real impact of the contact of calcium silicate-based material as a root canal dressing and the dentinal wall needs to be studied in order to clarify a possible influence on the sealing capacity of sealers. Besides, another relevant clinical aspect of being mentioned is how much of this material will remain in contact with the dentin after the application of different removal protocols, so that it does not interfere with the sealing ability.

In terms of the physicochemical properties studied, the new calcium silicate-based material (Bio-C Temp) presented alkaline pH, high calcium release, and acceptable radiopacity. Moreover, it presented a dose and time-dependent cytotoxic effect. Finally, CLSM showed Bio-C Temp and Ultracal XS surrounding the canal perimeter but with no tubule penetration.

Resumo

Os objetivos deste estudo foram avaliar as propriedades físicas e químicas, citototoxidade e penetração tubular de uma nova medicação à base de silicato de cálcio. Para o teste de pH, e liberação de íons cálcio (1, 24, 72 e 168 h) foi usado medidor de pH e espectofotômetro colorimétrico, respectivamente. Avaliação da radiopacidade, seguiu a ISO 6876:2012). A citotoxicidade foi avaliada pela porcentagem de células viáveis usando o ensaio MTT. Imagens ilustrativas de penetração tubular foram obtidas usando microscopia confocal de varredura a laser (CLSM). Os dados de pH e liberação de cálcio foram analisados através do teste de Análise de Variância de duas vias e teste de Tukey. A radiopacidade foi avaliada usando o teste T de Student. Para a citotoxicidade foi empregada a Análise de Variância de uma via e teste de Tukey. Ambos os materiais apresentaram pH alcalino em todos os tempos experimentais. Os valores de pH da pasta de hidróxido de cálcio foram superiores à pasta biocerâmica em 1, 24 e 72 h (p<0,05). A liberação de cálcio da pasta biocerâmica foi inferior à pasta de hidróxido de cálcio apenas em 24 h (p<0,05). Bio-C Temp foi mais radiopaco que o Ultracal XS (p<0,05). A pasta biocerâmica apresentou efeito citotóxico dependente da dose e do tempo de exposição. Imagens de CLSM mostraram ausência de penetração intratubular para ambas as pastas. A nova medicação à base de silicato de cálcio apresentou pH alcalino, alta liberação de cálcio e boa radiopacidade. Bio C Temp apresentou um efeito citotóxico dependente da dose e do tempo de exposição e ausência de penetração tubular.

References

1. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-1301.
2. Pereira TC, da Silva Munhoz Vasconcelos LR, Graeff MSZ, Ribeiro MCM, Duarte MAH, de Andrade FB. Intratubular decontamination ability and physicochemical properties of calcium hydroxide pastes. Clin Oral Invest 2019;23:1253-1262.
3. Vera J, Siqueira JF, Ricucci D, Loghin S, Fernández N, Flores B, et al. One- versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod 2012;38:1040–1052.
4. Zancan RF, Vivan RR, Milanda Lopes MR, Weckwerth PH, de Andrade FB, Ponce JB, et al. Antimicrobial activity and physicochemical properties of calcium hydroxide pastes used as intracanal medication. J Endod 2016;42:1822–1828.
5. Farhad A, Mohammadi Z. Calcium hydroxide: a review. Int Dent J 2005;55:293–301.
6. Verma R, Fischer BI, Gregory RL, Yassen GH. The radiopacity and antimicrobial properties of different radiopaque double antibiotic pastes used in regenerative endodontics. J Endod 2018;44:1376–1380.
7. Hench LL. Bioceramics: From Concept to Clinic. J Am Ceramic Soc 1991;74:1487–1510.
8. Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part II: other clinical applications and complications. Int Endod J 2018;51:284–317.
9. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dent Mater 2015;31:351–370.
10. Candeiro GT de M, Correia FC, Duarte MAH, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a Bioceramic root canal sealer. J Endod 2012;38:842–845.
11. Dahl JE, Frangou-Polyzois MJ, Polyzois GL. In vitro biocompatibility of denture relining materials. Gerodontology 2006;23:17–22.
12. Khalil I, Naaman A, Camilleri J. Properties of tricalcium silicate sealers. J Endod 2016;42:1529–1535.
13. Só MVR, da Silva LAB, Figueiredo JAP, Prochnow E. Calcium hydroxide

pastes: pH values and release of calcium ions. Italian Endod J 2005;19:235–238.

14. Okabe T, Sakamoto M, Takeuchi H, Matsushima K. Effects of ph on mineralization ability of human dental pulp cells. J Endod 2006;32:198–201.

15. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A Comprehensive literature review - Part I: chemical, physical, and antibacterial properties. J Endod 2010;36:16–27.

16. Vogel GL, Chow LC, Brown WE. A microanalytical procedure for the determination of calcium, phosphate and fluoride in enamel biopsy samples. Caries Res 1983;17:23-31.

17. Badole GP, Warhadpande MM, Meshram GK, Bahadure RN, Tawani SG, Tawani G, et al. A comparative evaluation of cytotoxicity of root canal sealers: an in vitro study. Restor Dent Endod 2013;38:204-209.

18. Mendes AT, Silva PBD, Só BB, Hashizume LN, Vivan RR, Rosa RAD, et al. Evaluation of physicochemical properties of new calcium silicate-based sealer. Braz Dent J 2018; 29:536–540.

19. Wang P, Henning SM, Heber D. Limitations of MTT and MTS based assays for measurement of antiproliferative activity of green tea polyphenols. PLoS One 2010;5:e10202.

20. Wang S, Yu H, Wickliffe JK, Limitation of the MTT and XTT assays for measuring cell viability due to superoxide formation induced by nano-scale TiO2. Toxicol In Vitro 2011;25:2147-2151.

21. van Tonder A, Joubert AM, Cromarty AD. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes 2015;8:47.

22. Paredes RM, Etzler JC, Watts LT, Zheng W, Lechleiter JD. Chemical calcium indicators. Methods 2008;46:143–151.

23. Jeong JW, DeGraft-Johnson A, Dorn SO, Di Fiore PM. Dentinal tubule penetration of a calcium silicate-based root canal sealer with different obturation methods. J Endod 2016;43:633-637.

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