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Physicochemical Properties, Cytocompatibility and Antibiofilm Activity of a New Calcium Silicate Sealer

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The aim of this study was to evaluate the physicochemical properties, cytocompatibility and antibiofilm activity of a new calcium silicate-based endodontic sealer, Sealer Plus BC (MK Life, Brazil), in comparison with TotalFill BC Sealer (FKG Dentaire SA, Switzerland) and AH Plus (Dentsply, Germany). Setting time and flow were evaluated based on ISO 6876 standard. The pH was evaluated after different periods, and radiopacity by radiographic analysis (mmAl). Solubility (% mass loss) and volumetric change (by micro-CT) were assessed after 30 days of immersion in distilled water. Cytocompatibility was assessed by methyltetrazolium (MTT) and neutral red (NR) assays, after exposure of Saos-2 cells to the sealer extract for 24 h. An additional analysis was performed by using MTT assay after 1, 3 and 7 days of exposure of Saos-2 to the sealers 1:8 dilution extracts. Antibiofilm activity against *Enterococcus faecalis* and/or *Candida albicans* was evaluated by crystal violet assay and modified direct contact test. The physicochemical properties were analyzed using ANOVA/Tukey tests; MTT and NR data were analyzed by ANOVA and Bonferroni tests; the antimicrobial tests were analyzed by Kruskal-Wallis and Dunn tests (α=0.05). Sealer Plus BC had proper setting time, radiopacity, flow and alkalization capacity. Sealer Plus BC was significantly more soluble than AH Plus ($p<0.05$) and presented volumetric change similar to AH Plus and TotalFill BC ($p>0.05$). Sealer Plus BC presented antibiofilm activity and no cytotoxic effect. In conclusion, although Sealer Plus BC had higher solubility, this sealer showed proper physicochemical properties, cytocompatibility, and antibiofilm activity.

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Introduction

Root canal filling should promote sealing avoiding reinfection (1), which is associated with treatment failure (2). Therefore, as endodontic sealers play an important role in the success of endodontic treatment (3), they must have volumetric stability (4) and antimicrobial activity (2, 5). The development of bioceramic and the introduction of new calcium silicate–based endodontic materials contribute to a better prognosis of endodontic treatment (3).

Premixed, ready-for-use bioceramic endodontic sealers have been developed showing adequate biological and physicochemical properties, with similar or better results than conventional endodontic materials (6). TotalFill BC Sealer (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is a root canal sealer containing calcium silicates with proper biological and physicochemical properties (7) besides antimicrobial effect (5). AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is an epoxy resin-based sealer considered as gold standard to comparison with new root canal sealers (4).

The main advantage of AH Plus in comparison with calcium silicate-based sealers is its low solubility (6). However, the conventional test used to evaluate this property may not reproduce a clinical condition (4). Microcomputed tomography (micro-CT) has been used to evaluate root canal sealer properties (4, 7). As a 3D non-destructive technique, micro-CT results in volumetric reconstruction allowing to measure variables on the same specimen before and after experimental periods (8). This imaging method allows to evaluate the solubility and dimensional changes of endodontic sealers after immersion in a fluid by evaluating the volumetric change of materials (1, 7, 9). The volumetric change test is suitable for evaluating materials with fluid uptake, such as calcium silicate-based sealers (1, 7, 9).

Recently, a new calcium silicate-based sealer, Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil)

was developed. This material has proper physicochemical properties, except for a high solubility (10). Sealer Plus BC also showed low cytotoxicity and more biocompatibility than MTA Fillapex (Angelus, Londrina, PR, Brazil) and AH Plus (11). However, there are no studies considering the antimicrobial properties of Sealer Plus BC, and there is no evaluation of the volumetric change of this material after immersion in distilled water for 30 days.

The aim of this study was to evaluate physicochemical properties (pH, setting time, radiopacity, flow, solubility and volumetric change), cytocompatibility and antibiofilm activity of calcium silicate-based sealers (TotalFill BC and Sealer Plus BC) in comparison with an epoxy resin-based sealer (AH Plus). The null hypothesis was that there is no difference between the sealers in the evaluated physicochemical properties, cytocompatibility and antibiofilm activity against *E. faecalis* and/or *C. albicans*.

Materials and Methods

The endodontic sealers and their respective manufacturers, composition and proportion used are described in Table 1.

Table 1 - Endodontic sealers, manufacturer, composition and proportion used

Material	Manufacturer	Composition	Proportion
Sealer Plus BC	MK Life, Porto Alegre, RS, Brazil	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, and propylene glycol.	Ready to use
TotalFill BC	FKG Dentaire SA, La Chaux-de-Fonds, Switzerland.	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.	Ready to use
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany.	Bisphenol A/F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments dibenzyldiamine, aminoadamantane, silicone oil.	1 g : 1 g (Paste/Paste)

Physicochemical properties

Setting time

Plaster models with cavities 10 mm in diameter and 1 mm deep (n = 6) were manufactured as recommended by ISO 6876:2012 (12) to assess the setting time of materials which need moisture for setting. Although AH Plus is an epoxy resin-based sealer, a previous test showed values similar to those obtained by evaluating this sealer in metal rings. Therefore, in order to standardize the assessment, type IV plaster molds (Dentsply, Petrópolis, Rio de Janeiro, Brazil) were manufactured based on a previous study (9). The molds were kept immersed in distilled water for 24 hours at 37ºC before the test (12). Then, the cavities were filled with the sealers. A Gilmore needle with mass of 100 ± 0.5 g and diameter of 2 ± 0.1 mm was used. The molds with the sealers were kept in an oven (37 ± 1 °C, 95 ± 5% relative humidity). The setting time was considered as the time when the marks of needle could not be observed on the sealer surface.

Radiopacity

Specimens (10 mm in diameter and 1 mm height) were made (n = 6 per group). Each specimen was positioned on occlusal radiographic films (Insight-Kodak Comp, Rochester, NY, USA) and exposed, along with an aluminium step wedge with variable thickness (from 2 to 16 mm, in 2-mm increments). An X-ray unit (Instrumentarium Dental, Tuusula, Finland) operating at 60 kV, 7 mA, 0.32 pulses per second, and focus-film distance of 33 cm was used. The films were processed and digitized. The images were imported to the Image Tool 3.0 software (UTHSCSA, San Antonio, TX, USA); an equal-density tool was used to identify equal-density areas in the radiographic images. The values recorded for each material were averaged to obtain a single value in mmAl.

Flow

The flow test was realized in accordance with ISO standard 6876:2012 (12). After manipulation, 0.05 ± 0.005mL of the sealer was placed on a glass plate (n = 10 per group). At 180 ± 5 seconds after initiating the manipulation, another glass plate (20 g) was placed on the plate with the sealer, and a 100 g weight was put on the top plate, and kept there for 10 minutes. The maximum and minimum diameters of the material on glass plate were measured. When a difference of less than 1 mm between the diameters was observed, the mean value was recorded. A second analysis was made by photographing the material on the plate alongside a millimetre ruler. The images were imported to the Image Tool 3.0 software and the area of flow of the material was expressed in mm^2.

pH

Polyethylene tubes measuring 10 mm length and 1.6 mm diameter were filled with freshly sealer (n = 10 per group). The tubes were immersed in plastic flasks containing 10 mL of deionized water. The flasks were closed and kept in an oven at 37º C. pH measurements were made after time intervals of 1, 7, 14, and 21 days. The solutions pH was analysed at each period using a previously calibrated digital pH meter (Digimed, São Paulo, SP, Brazil). The mean pH value (in triplicate) in each experimental period was calculated.

Solubility

Solubility evaluation (n = 6) was performed based on previous studies (4, 7, 9). Specimens measuring 1.5 mm high and 7.75 mm in diameter were manufactured (13). A nylon thread was inserted in the fresh sealer and the molds were kept at 37°C and 95% humidity for three times the setting time of sealers (7, 9). The sealers were removed from the molds and weighed on a balance (Adventurer - Ohaus, Model AR2140 - Indústria de Balanças Ltda., São Bernardo do Campo, SP, Brazil). Then, each specimen was placed in a closed plastic flask containing 7.5 mL of distilled and deionized water (2, 6, 9). The sealers were attached to the containers with nylon threads and kept in an oven at 37°C for 30 days (4, 7, 9). After this period, they were washed in distilled water, and placed in a dehumidifier containing silica until the mass stabilization. Mass was measured before and after the samples were immersed in distilled water, and every 24 hours thereafter, until the mass stabilization (approximately 7 days). The difference between the initial and final weights was recorded as the materials solubility. This difference in mass was transformed into a percentage based on the initial weight (% mass loss).

Volumetric change

This test was performed based on a previous study (9). The specimens (n = 6) were prepared with the same dimensions as those used for the solubility test, but with no nylon thread. After setting, the sealers were scanned by micro-computed tomography SkyScan 1176 (Bruker-MicroCT, Kontich, Belgium). After initial scanning, the sealers were immersed in plastic flasks containing 7.5 mL of distilled water and were kept in an oven for 30 days. After 15 days, the position of the sealers in the flask was changed in order to allow the contact with the water in both superficies of the sealer for the same time. Then, the sealers were placed in a desiccator containing silica for 24 hours and scanned again. The scanning parameters for all materials evaluated were: voltage of 80 kV, 300 μA current, at 18 μm voxel size, copper and aluminium (Cu + Al) filter and 360° rotation. These settings were based on Zordan-Bronzel et al. (9) and confirmed an initial test. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker-MicroCT, Kontich, Belgium). The correction parameters for smoothing, beam hardening, and ring artefacts were defined for each sealer. The parameters for Sealer Plus BC and TotalFill BC Sealer that have similar composition were: 1 for smoothing, 61 for beam hardening correction and 1 for ring artefacts correction; and for AH Plus were 1 for smoothing, 21 for beam hardening correction and 1 for ring artefacts correction. The reconstructed images were superimposed using the Data Viewer software (V1.5.2.4; Bruker-MicroCT, Kontich, Belgium). The 3D images were used for quantitative analysis of the samples, allowing the total volume of sealer to be calculated in mm^3 by CTAn software (V1.15.4.0; Bruker-MicroCT, Kontich, Belgium). The volumetric change between the baseline and the experimental period was calculated.

Cell viability assays

Cell viability was evaluated by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and neutral red (NR) assays (14). Human osteoblast-like cells, Saos-2 (ATCC HTB-85), were

cultivated in T-75 flasks (Jet Biofil, Guangzhou, China), containing DMEM (Dulbecco modified medium; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco/Invitrocell, Life Technologies, Grand Island, NY, USA), penicillin (100 IU/mL), and streptomycin (100 µg/mL), until confluence. AH Plus (Dentsply DeTrey, Konstanz, Germany) was used as the reference material. For the preparation of extracts, 0.5 g of each evaluated sealer was placed in the bottom of empty wells of 12-well culture plates. The plates were stored at 37 °C, 95% humidity and 5% CO2, for 24 hours, until the complete setting of the materials. After this period, the plates were exposed to ultraviolet light to prevent contamination. Then, 5 mL of DMEM serum-free was added in each of the plate wells. The plates were kept at 37°C for 24 hours, in 95% humidity and 5% CO_2. Then, the dilutions of the sealer extracts were prepared (1:1, 1:2; 1:4; 1:8, 1:16 and 1:32).

MTT assay

Saos-2 cells were plated (1 x 10^5 cells mL^{-1}) in 96-well plates (Jet Biofil) containing DMEM supplemented with 10% FBS (Gibco), penicillin (100 IU/mL), streptomycin (100 µg/mL). Cells were cultured for 24 hours at 37 °C, 95% humidity and 5% CO_2 before exposure to the materials. Saos-2 were exposed to sealer extracts at 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions, negative control (serum-free DMEM) and the positive control, 20% dimethyl sulfoxide (DMSO) for 24 hours, according to ISO 10993 (15). Additionally, the viability of cells exposed to the sealer extracts in the 1:8 dilution was analyzed through MTT assay performed for the experimental periods of 1, 3 and 7 days. The extracts were renewed every 48 hours. Immediately after each trial period, the medium was replaced with 100 µL of MTT 5 mg mL^{-1} solution (Sigma-Aldrich) and the plates were incubated for 3 h. Isopropanol (100 µL) acidified to 0.04 N (Sigma-Aldrich) was added. Optical density was measured at 570 nm in a spectrophotometer UVM 340 (ASYS, Nova Analítica, São Paulo, SP, Brazil).

Neutral red (NR) assay

The same procedures described in the MTT assay were performed. After 24-hour exposure of cells to the sealer extracts, the extracts were replaced with 100 µL of DMEM containing 50 µg NR mL^{-1} (Sigma-Aldrich) and the plates were incubated for 3 hours. The colorimetric product was solubilized in solution (50% ethanol and 1% acetic acid) (Sigma-Aldrich). Optical density was measured in a spectrophotometer at 570 nm. Three independent experiments were performed in triplicate for each experimental group, and the mean of each experiment was used in the statistical analysis (n = 3 per group).

Antibiofilm activity

Crystal violet assay

The action against the biomass of mono- and dual-species biofilms of *E. faecalis* (ATCC 29212) and/or *C. albicans* (ATCC 10231) was evaluated by violet crystal assay. The biofilms (adjusted to 1 x 10^8 CFU mL^{-1}) were formed in 96-well plates for 72 hours (*E. faecalis*) and 48 hours (*C. albicans* and dual-species biofilms), with Tryptic soy broth – TSb culture medium (Difco Detroit, MI, USA) supplemented with D-(+)-Glucose (Sigma-Aldrich) for *E. faecalis* ; and in Brain Heart Infusion – BHI (Difco) culture medium for *C. albicans* and dual-species biofilms. The sealers were manipulated and placed in contact with 2 mL of saline solution, in an oven, at 37°C for 48 hours to obtain the extracts. After biofilm formation, the wells were washed three times with 200 µL of PBS 1x (pH 7.2) and 200 µL of the extracts were applied in each well (n = 8 per group), and incubated at 37°C for 24 hours. After this time, the extracts were removed and each well was washed with PBS 1x. The plates were dried at room temperature for 24 hours. Afterwards, the wells were stained with 200 µL of 0.1% crystal violet solution (Synth, Diadema, SP, Brazil) at room temperature for 20 minutes. The excess stain was rinsed by washing with distilled water. The plates were dried at room temperature and the dye linked to the adherent cells was solubilized with 200 µL of 33% acetic acid. To quantify the biofilm biomass remaining after treatment, the absorbance was measures (590 nm). For the positive control (PC), saline solution was used, and the negative control was sterile culture medium. Data were expressed in percentage of biomass reduction.

Modified direct contact test (MDCT)

The modified direct contact test was performed based on previous studies (2, 14). Sterilized bovine root dentin slices (n = 6 per group) with a size of 5 mm x 5 mm x 0.7 mm (width x length x thickness) were submersed in TSb (2 mL) containing 1% of the inoculum of *E. faecalis* (1 x 10^8 CFU mL^{-1}), in 24-

well culture plates. The plates were kept in a microaerophilic environment for 14 days. Disks of each sealer were made, measuring 7 mm in diameter x 1 mm in internal thickness, which were kept in an oven at 37 ° C and controlled humidity for 48 hours. Then, the disks of each sealer were placed on dentin slices containing the formed biofilm. In the positive control group, a teflon disc was used. Time of contact was 15 hours. The dentin slices were placed individually in microtubes containing 1 mL of saline solution and glass pearls, and they were agitated in a vortex for 1 minute (Model Q220, Quimis Aparelhos Científicos Ltda., Diadema, SP, Brazil). Afterwards, serial decimal dilution was performed, and the antibiofilm activity of the sealers was assessed by a colony-forming unit (CFU) counting method. The data were submitted to logarithmic transformation (log10).

Statistical analysis

All data was analysed with the GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA) statistical software package. Physicochemical data were analysed by one-way analysis of variance (ANOVA) and post hoc Tukey's tests. Cell viability were analyzed by two-way ANOVA test with Bonferroni correction. The antimicrobial tests were analyzed by Kruskal-Wallis and Dunn tests (α=0.05).

Results

The results of physicochemical properties are represented in Table 2. Setting time of Sealer Plus BC was shorter than TotalFill BC and AH Plus ($p<0.05$). Sealer Plus BC presented proper radiopacity and greater flow by area analysis. Sealer Plus BC presented similar flow to TotalFill BC (diameter analysis). At 1 day, the pH values were greater for TotalFill BC, followed by Sealer Plus BC ($p<0.05$). No difference was observed between TotalFill BC and Sealer Plus BC in the other periods ($p>0.05$). AH Plus was similar to control (distilled water) at all periods ($p>0.05$). Solubility was greater for TotalFill BC when compared with other sealers ($p<0.05$). AH Plus had the lowest solubility ($p<0.05$). Regarding volumetric change, all sealers had a volume decrease. Sealer Plus BC had similar values than other sealers ($p>0.05$).

Table 2 - Mean and standard deviation of setting time, radiopacity, flow, pH, solubility, and volumetric change observed in the different root canal sealers.

Tests		AH Plus	TotalFill BC	Sealer Plus BC	Control (H_2O)
Setting time (min)		383.8 (±5,31)^{b}	580.2 (±21.74)^{a}	195.0 (±24.29)^{c}	-----
Radiopacity (mmAl)		9.11 (±0.49)^{a}	6.09 (±0.80)^{b}	4.17 (±0.28)^{c}	-----
Flow (mm)		20.99 (±0.86)^{b}	24.92 (±1.31)^{a}	25.60 (±1.08)^{a}	-----
Flow area (mm^2)		402.9 (±98.62)^{c}	525.1 (±47.25)^{b}	695.6 (±67.90)^{a}	-----
pH	1 day	6.49 (±0.11)^{c}	10.46 (±0.20)^{a}	9.83 (±0.31)^{b}	6.35 (±0.29)^{c}
	7 days	6.17 (±0.71)^{b}	10.27 (±0.56)^{a}	9.76 (±0.68)^{a}	6.74 (±0.14)^{b}
	14 days	6.60 (±0.35)^{b}	10.50 (±0,51)^{a}	9.66 (±0.74)^{a}	6.27 (±0.33)^{b}
	21 days	6.11 (±0.26)^{b}	9.65 (±0.93)^{a}	9.98 (±0.57)^{a}	6.51 (±0.45)^{b}
Solubility (% mass loss)		0.33 (±0.20)^{c}	10.09 (±2.58)^{a}	4.71 (±1.27)^{b}	-----
Volumetric change (%)		-0.41 (±0.20)^{b}	-1.98 (±1.09)^{a}	-1.19 (±0.56)^{ab}	-----

Different letters on the same line indicate statistically significant difference ($p<0.05$).

In the MTT assay, Sealer Plus BC in the 1:1 and 1:2 dilutions had significantly lower cell viability (51.14% and 84.53%, respectively) compared to the other sealers and the negative control ($p<0.05$) (Figure 1). NR assay revealed that AH Plus, Sealer Plus BC and TotalFill BC had no cytotoxic effects on Saos-2 cells, as cell viability not significantly different when compared to the negative control ($p>0.05$) (Figure 1). In the periods of 1 and 7 days (Figure 2), Sealer Plus BC showed greater cell viability in comparison to the control group ($p<0.05$).

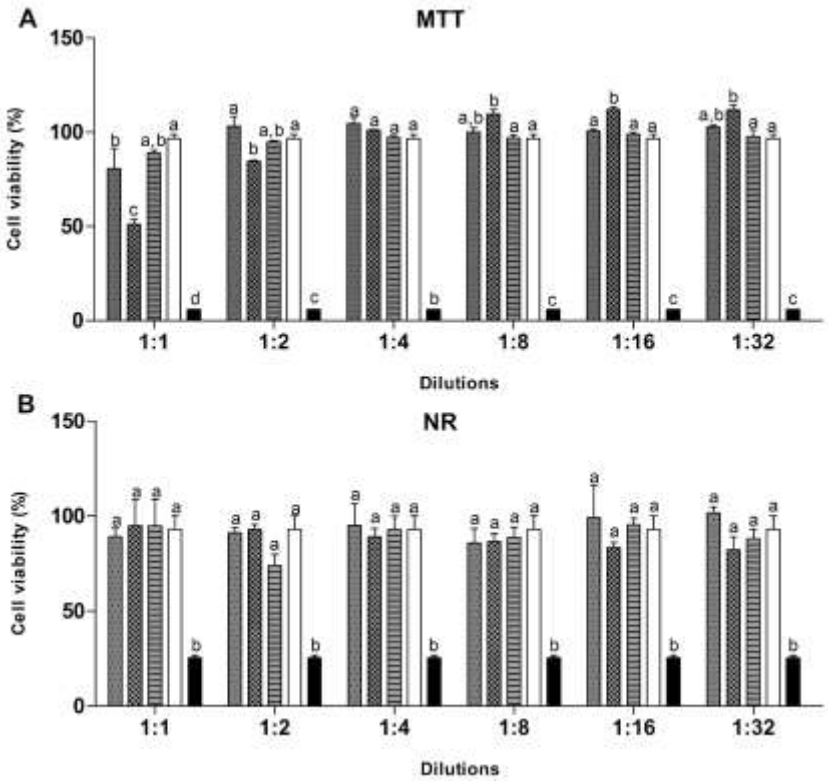

Figure 1 - Saos-2 cell viability evaluated by MTT assay after exposure to AHP, SPBC and TFBC sealers extracts at 1:2, 1:4, 1:8, 1:16 and 1:32 dilutions, serum-free culture medium (negative control), and 20% DMSO (positive control). Bars with different letters represent significant differences between sealers (in each dilution), negative control and positive control ($p<0.05$). AHP- AH Plus; SPBC- Sealer Plus BC; TFBC- TotalFill BC Sealer; NC- negative control; PC- positive control.

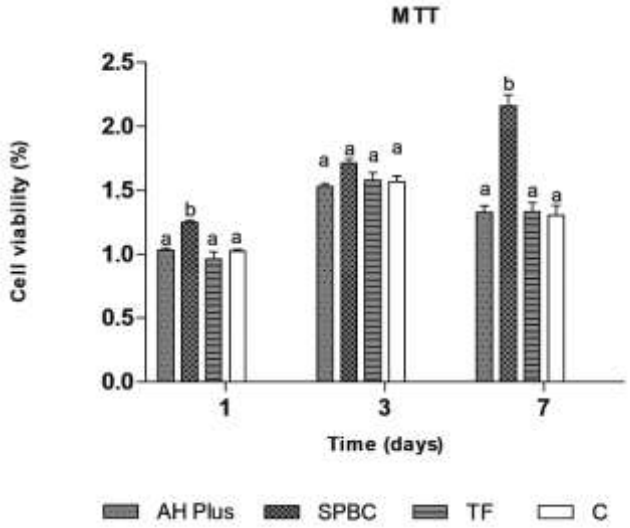

Figure 2 - Saos-2 cell viability evaluated by MTT assay after exposure to AHP, SPBC, TF at 1:8 dilution and serum-free culture medium (negative control), for the time intervals of 1, 3 and 7 days. Bars with different letters represent significant differences between sealer extracts and negative control in each period. AHP- AH Plus; SPBC- Sealer Plus BC; TF, TotalFill BC Sealer; C, negative control.

In the crystal violet assay, Sealer Plus BC and TotalFill BC had significantly greater antibiofilm activity against *E. faecalis* biofilm when compared to AH Plus ($p<0.05$), without difference between them ($p>0.05$) (Table 4). Sealer Plus BC was more effective against *C. albicans* ($p<0.05$). No significant difference in the biomass reduction was observed amongst the sealers ($p>0.05$) against dual-species biofilm (Table 3). In the MDCT, TotalFill BC was more effective than other sealers, followed by Sealer Plus BC ($p<0.05$). No significant difference was observed amongst AH Plus and positive control ($p>0.05$).

Table 3 - Mean and standard deviation of antimicrobial activity of the materials evaluated, expressed in CFU mL^{-1} for modified direct contact test on *E. faecalis* biofilm, after 15 hours of contact

	AH Plus	**TotalFill BC**	**Sealer Plus BC**	**Positive control**
CFU mL^{-1}	7.413 (±0.09)[a]	4.789 (±0.44)[c]	5.814 (±0.53)[b]	7.583 (±0.27)[a]

Different letters on the same line indicate statistically significant difference ($p<0.05$).

Table 4 - Mean and standard deviation of percentage biomass reduction after 24 hours in contact with sealer extracts (48 hours) with mono- and dual-species biofilms of *E. faecalis* and/or *C. albicans*

Strain	**AH Plus**	**TotalFill BC**	**Sealer Plus BC**
E. faecalis	15.90 (±4.1)[b]	52.48 (±6.8)[a]	57.54 (±4.2)[a]
C. albicans	12.20 (±4.8)[c]	52.31 (±6.5)[b]	71.88 (±4.9)[a]
Dual-espécies	21.43 (±3.5)[a]	14.61 (±6.3)[a]	21.37 (±3.5)[a]

Different letters on the same line indicate statistically significant difference ($p<0.05$).

Discussion

Based on the obtained results, our null hypothesis was partially rejected since in general the sealers showed different physicochemical properties. Overall, both calcium silicate-based materials were associated with flow, solubility and pH greater than AH Plus. However, some differences were also observed between TotalFill BC and Sealer Plus BC. The new sealer when compared to TotalFill BC has shorter setting time, better flow when evaluating in mm^2, lower solubility, and its volumetric change was similar to AH Plus.

Regarding the setting time of the sealers, shorter setting time was also observed to AH Plus when compared with TotalFill BC (7). AH Plus also showed longer setting time than Sealer Plus BC (10). However, the values of the setting time of AH Plus and Sealer Plus BC differ from a previous study (10). This difference may be related to the fact that the present study evaluated the setting time according to ISO 6876:2012, while Mendes et al. (10) evaluated the setting time according to ASTM C266-03 (16). ISO 6876 considers setting time as the time from sealers manipulation until the moment when the marks of the Gilmore needle could not be observed on the sealer surface (12). ASTM C266 in addition to recommending the evaluation of initial setting time and final setting time, considers that the initial setting time is the time between the initial contact of the sealer and the liquid, and the time when the initial Gilmore needle does not leave a complete circular impression on the surface of the cement (16). Another difference between these methods is related to the Gilmore needle used. ISO 6876 recommends the use of a Gilmore needle with a mass of 100 ± 0.5 g and diameter of 2 ± 0.1 mm (12, 17), while ASTM C266 recommends the use of a Gilmore needle with a mass of 113.4 ± 0.5 g and diameter of 2.12 ± 0.05 mm (16). Although Sealer Plus BC has the shortest setting time, this time is adequate for its clinical applicability.

According to ISO 6876 standard (12), the radiopacity of a root canal filling material should be greater than 3 mm Al. This property is important in order to distinguish the sealer from the anatomical structures (10). The flow ability of root canal sealer allows sealer to penetrate into the irregularities,

isthmus and ramifications of root canal system (18). Moreover, the obturation should provide filling of the irregular areas of the root canal (19). All the evaluated sealers presented radiopacity and flow in accordance to the ISO standard, in agreement with previous studies (7, 10).

Solubility and pH after long periods are evaluated to analyze the behaviour of sealers over time (2, 9). The alkalinization ability contributes to the repair process by mineralized tissues (17). The pH and solubility of cements can be related, since solubility can contribute to the release of hydroxyl ions (18). Calcium silicate sealers promoted high pH and high solubility. This result may be related to the hydrophilic property of materials based on calcium silicate, which present greater solubility when evaluated by immersion in water. The alkaline pH is explained by the release of OH^- and Ca^{2+} ions and can play an important role in the repair of periapical tissues (19). AH Plus did not promote an increase in pH as observed for epoxy resin-based materials (18). AH Plus is hydrophobic and has no water absorption; consequently, its solubility is significantly lower (20).

Sealer Plus BC and TotalFill BC Sealer have a similar composition. Although the high solubility of Sealer Plus BC and TotalFill BC Sealer may appear to be a disadvantage of calcium silicate-based sealers (19), the tests available considering only mass loss after immersion in water may be inadequate for evaluating these materials (20). In order to compensate the limitations of conventional tests, micro-CT have been proposed as an alternative to evaluate solubility and dimensional changes of sealers by means of the volumetric change of the materials after immersion in a fluid (1, 7). Our findings showed that even with greater values of mass loss, TotalFill BC and Sealer Plus BC showed low volumetric reduction. So, we can assume that the solubility of these sealers could be compensated by their fluid absorption, resulting in a volumetric stability (1). These results suggest that volumetric change test present more similarities with clinical performance of root canal sealers (7).

Cell viability after exposure to the different dilutions of sealer extracts was evaluated by MTT and NR assays in human Saos-2 osteoblast cells (14). Benetti et al. (20) observed that the Sealer Plus BC extract (at 1:50 dilution) had higher cytocompatibility on L929 fibroblast in comparison to MTA Fillapex and AH Plus sealers, and exhibited reduction in cell viability at 1:100 and 1:200 dilutions. In the analysis performed for 1, 3 and 7 days, it was observed that AH Plus, Sealer Plus BC and TotalFill BC Sealer demonstrated cytocompatibility with Saos-2 cells. Moreover, there was a decrease in the cell viability after 7 days with the exception of Sealer Plus BC. Our results corroborate a previous study, which reported that there was a decrease in cell viability in the MTT assay, when cells were tested with DMEM culture medium (Sigma/Aldrich) serum-free (without the presence of FBS) (21). The serum-free medium has nutrients, growth factors, hormones, trace elements and factors that promote adherence (21), and it does not present the undesired effect of FBS, which can interfere with the results of the MTT assay (22).

Although several microorganisms are frequently associated with endodontic infections (5), antimicrobial activity of endodontic sealers is usually evaluated against *E. faecalis* and *C. albicans*, since these microorganisms are commonly observed as biofilm, increasing their resistance to antimicrobial agents when compared with the planktonic microorganisms (5, 23). *E. faecalis* is associated with failure of endodontic treatment (2). *C. albicans* is a fungal specie observed in isolated pure strains or in association with Gram-positive and negative bacteria, and has the capacity to survive in severe environmental conditions (23).

The direct contact test (DCT) is an established and widely used methodology for the evaluation of antimicrobial activity (2, 5). However, materials are usually evaluated against bacteria in planktonic form. The modified direct contact test (MDCT) used in the present study evaluate materials in direct contact with biofilm. A previous study considered the evaluation of endodontic materials after setting as a disadvantage for MDCT (2). However, calcium silicate-based endodontic materials have significant antimicrobial activity even after long periods, such as 30 days (6). Therefore, the analysis in the present study sealers was performed 48 hours after manipulation of sealers.

In the present study, Sealer Plus BC had antimicrobial activity against *E. faecalis* biofilm, but was more effective against *C. albicans*. TotalFill BC had antimicrobial activity against *E. faecalis* and/or *C. albicans* biofilms. TotalFill BC demonstrated greater effectiveness against *E. faecalis* when compared to AH Plus, corroborating a previous study (5). The alkalinization capacity of calcium silicate-based sealers may be related to their antimicrobial activity.

The techniques used for cleaning and disinfecting of root canals are effective in reducing the microbial load, but they are not able to eliminate microorganisms (5). Dimensional change of endodontic sealers may compromise the sealing of the root canal (4), allowing reinfection (1). Since Sealer Plus BC

has volumetric stability and antimicrobial activity, it can contribute to the success of endodontic treatment.

An important limitation of the current study is that results may not represent a clinical situation. In addition, we must also take into account that international standards for assessment of root canal sealers may not be ideal, mainly when evaluating calcium silicate-based sealers regarding setting time and solubility (24). However, the results of basic research protocols using in vitro studies can contribute to a better understanding of the behavior of bioceramic materials (25).

In conclusion, Sealer Plus BC has cytocompatibility, antibiofilm activity against *E. faecalis* and/or *C. albicans*, and physicochemical properties as setting time, flow, radiopacity, volumetric change and pH suitable for clinical use. However, this endodontic sealer showed solubility above than that recommend by ISO 6876. Further in vivo and clinical researches should be performed to assess new bioceramic sealers.

Declaration of conflict of interest: The authors declare that they have no conflict of interest.

Resumo

O objetivo deste estudo foi avaliar as propriedades físico-químicas, a citocompatibilidade e a atividade antibiofilme de um novo cimento endodôntico à base de silicato de cálcio, Sealer Plus BC (MK Life, Brasil), em comparação com TotalFill BC Sealer (FKG Dentaire SA, Suíça) e AH Plus (Dentsply, Alemanha). O tempo de presa e o escoamento foram avaliados com base nas normas ISO 6876. O pH foi avaliado após diferentes períodos, e a radiopacidade por análise radiográfica (mmAl). A solubilidade (% de perda de massa) e alteração volumétrica (por micro-CT) foram avaliadas após 30 dias de imersão em água destilada. Citocompatibilidade foi avaliada pelos ensaios metiltetrazólio (MTT) e vermelho neutro (NR), após exposição das células Saos-2 ao extrato de cimento por 24 horas. Análise adicional foi realizada através do ensaio MTT após 1, 3 e 7 dias de exposição das células Saos-2 aos extratos dos cimentos na diluição de 1:8. Atividade antibiofilme contra *Enterococcus faecalis* e/ou *Candida albicans* foi avaliada pelos ensaios cristal violeta e contato direto modificado. As propriedades físico-químicas foram analisadas utilizando os testes ANOVA e Tukey; MTT e NR foram analisados pelos testes ANOVA e Bonferroni; os ensaios antimicrobianos foram analisados pelos testes Kruskal-Wallis e Dunn (α=0.05). Sealer Plus BC apresentou tempo de presa, radiopacidade e escoamento adequados, além de capacidade de alcalinização. Sealer Plus BC foi significantemente mais solúvel que AH Plus ($p<0.05$) e apresentou alteração volumétrica similar à de AH Plus e TotalFill BC ($p>0.05$). Sealer Plus BC apresentou atividade antibiofilme, sem efeito citotóxico. Como conclusão, embora Sealer Plus BC apresente maior solubilidade, este cimento apresentou propriedades físico-químicas adequadas, citocompatibilidade e atividade antibiofilme.

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