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Calcium Silicate-Based Experimental Sealers: Physicochemical Properties Evaluation

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The aim of this study was to evaluate physicochemical properties of calcium silicate-based experimental (CS) endodontic sealers, developed using two different vehicles: polyethylene glycol (PG) (CS-PG) or polyethylene glycol associated to chitosan hydrogel (CS-PGCH). TotalFill BC Sealer (TF) and AH Plus (AHP) were evaluated for comparison. Setting time, flow, radiopacity, pH, solubility and volumetric change were analyzed. Data were submitted to ANOVA and Tukey tests with 5% significance level. The CS-PGCH had significantly greater setting time. CS-PG flow was similar to AHP. CS-PG had higher radiopacity than CS-PGCH. Calcium silicate-based sealers presented alkaline pH in all periods. CS-PGCH presented higher solubility in comparison with CS-PG. The volumetric change of CS-PG was similar to TF after 7 days, and to AHP after 30 days. CS-PG presented proper setting time, radiopacity, flow and pH, besides low volumetric change, showing better results than CS-PGCH, and potential for clinical application.

Keywords: *Biocompatible Materials, Calcium Silicate, X-Ray Microtomography, Dental Materials, Physical Properties.*

1. Introduction

Calcium silicate-based materials were developed as repair cements[1]. The presence of tricalcium silicate increases mechanical properties and bioactivity of the materials[2]. Currently, calcium silicate materials are widely used as root canal sealers[3,4]. However, in order to provide improved physicochemical properties and flow for calcium silicate materials, new formulations are proposed[1], using different vehicles to obtain adequate consistency for clinical applicability[5]. Polyethylene glycol is traditionally used as vehicle for calcium hydroxide pastes[6]. Bio-C Sealer is a new ready-to-use bioceramic endodontic sealer that contains polyethylene glycol as vehicle and presents biocompatibility, bioactive potential[7] and suitable physicochemical properties for clinical use[8]. EndoSequence BC (Brasseler, Savannah, GA, USA) and TotalFill BC (FKG, La Chaux-de-Fonds, Switzerland) are also premixed bioceramic sealers, which have similar composition[3,9]. These sealers present suitable properties[8,9], such as cytocompatibility[10], biocompatibility[11], bioactivity[12] and antimicrobial activity[13].

New root canal filling materials are developed, composed by calcium silicates, radiopacifying agents, and a vehicle to obtain ideal characteristics. A previous study[13] evaluating an experimental calcium silicate-based endodontic sealer with polyethylene glycol as vehicle showed cytocompatibility, bioactive potential, and antimicrobial activity, with potential for clinical application. In addition, since failure of endodontic therapy is related to the persistence of microorganisms in the root canal system[14,15], root canal sealers should present antimicrobial properties. The association of chitosan as vehicle can promote increased antimicrobial activity[16], inhibiting bacterial penetration and colonization[17]. This association can also favor the formation of hydroxyapatite[16] in addition to dentinal biomineralization[18].

ISO 6876 standard[19] is used for the evaluation of endodontic sealers. Since the physicochemical properties of the polyethylene glycol or chitosan as vehicles for calcium silicate-based endodontic sealers have not yet been studied, the aim of this study was to develop two experimental endodontic sealers based on tricalcium and dicalcium silicates and evaluate their physicochemical properties. The null hypothesis was that would be no difference among the sealers evaluated regarding these properties.

2. Experimental

2.1 Materials

The experimental sealers were developed using a powder formulation: tricalcium silicate (46.55%), dicalcium silicate (6.65%), monobasic calcium phosphate (5.00%), calcium hydroxide (3.80%) and zirconium oxide (38.00%). Five vehicle options were tested: Hydroxyethylcellulose (Natrosol), Polyethylene Glycol (PG), Chitosan hydrogel (CH), Carboxymethylcellulose, PG+CH. Based on handling property and setting time, two vehicle options were selected: PG, and

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the association PG/CH. However, the radiopacity presented by these formulations did not comply the specifications of ISO 6879:2012[19] (above 3 mm Al). Therefore, calcium tungstate was added to the base powder to improve the radiopacity. The final formulation was obtained: tricalcium silicate (46.55%), dicalcium silicate (6.55%), monobasic calcium phosphate (5.00%), calcium hydroxide (3.80%), zirconium oxide (28.50%) and calcium tungstate (9.50%). The handling of powder was performed with vehicle options. The proportion of the selected vehicles was determined after flow tests with different proportions.

After the preliminary tests, two experimental sealers were selected to be evaluated in comparison with AH Plus and TotalFill BC. The sealers, their manufacturers, composition and proportions are described in Table 1.

2.2 Setting time

The setting time (ST) was evaluated according to the ISO 6876:2012 specifications[19]. Type IV plaster molds (Dentsply, Petrópolis, Rio de Janeiro, Brazil) were made with an internal diameter of 10 mm and height of 1 mm (n = 6), and kept in distilled water for 24 hours. The materials were mixed, placed into plaster molds and stored in an oven under moisture control (37 ± 1 °C, 95 ± 5% relative humidity). A Gilmore needle with mass of 100 ± 0.5 g and diameter of 2 ± 0.1 mm was used for determination of the ST. The ST was considered as the period in minutes, between manipulation and the moment at which the needle did not leave indentation on the surface of the sealer, and the materials were stored in the oven throughout this period.

2.3 Flow

The flow was analyzed in accordance to the ISO 6876:2012[19] standard. After manipulation, 0.05 mL of the sealer was placed on a glass plate (n = 10). Then, another glass plate (20 g) was placed over the sealer together with a device weighing 100 g, for 10 minutes. Then, the shortest diameter and the longest diameter of the sealer disks were measured using a digital caliper. The mean value was recorded when a difference of less than 1.00 mm between the diameters was observed. Two methods were used to perform the measurements. For the second method, the specimens were photographed next to a ruler graduated in millimeters. The images obtained were digitized and the area of sealer was measured in mm^2 in the UTHSCSA Image Tool for Windows program, Version 3.00, as described by Tanomaru-Filho et al.[20].

2.4 Radiopacity

Specimens with an internal diameter of 10 mm and 1 mm height were made of the sealers (n = 6). After complete setting (37 ± 1 °C, 95 ± 5% relative humidity, 3 times the duration of their setting time), one disc of each material and an aluminum scale were placed on an occlusal film (Insight – Kodak Comp, Rochester, NY) to take a radiograph with a focus X-ray appliance (Instrumentarium Dental, Tuusula, Finland), operated at 60 kV, 7 mA, 18 pulses/s and focal distance of 33 cm. The films were processed, digitized, and analyzed using Image J for Windows software, in which areas of each of the steps of the millimeter scale were selected to determine the radiopacity equivalence of the sealers in millimeters of aluminum (mm Al). The values obtained were converted as described by Hungaro-Duarte et al.[21].

2.5 pH

Polyethylene tubes measuring 10 mm length and 1.6 mm in diameter were filled with each material (n = 10). Each tube was immersed in a plastic flask with 10 mL distilled water and stored in at 37 ± 1 ºC. For the control group, distilled water was used. After each experimental period, the tubes were placed in new flasks containing 10 mL of distilled water. The experimental periods were 3, 12 and 24 hours, 7, 14 and 21 days. The pH of the water in which the tubes had been kept was measured with a pH meter (model DM-21, Digimed, São Paulo, SP, Brazil), previously calibrated using buffer solutions of pH 4.01, 6.86, and 10.01 (Digimed).

2.6 Solubility

Solubility was determined based on Carvalho-Junior et al.[22] Test specimens (7.75 mm in diameter by 1.5 mm height) were made (n = 6), using silicone molds. An impermeable nylon thread was embedded in the fresh sealer mixture. A glass slab covered with cellophane film was placed over the molds. For calcium silicate-based sealers, pieces of damp gauze were placed between the molds and plates, according to a previous study[9]. After complete setting (37 ± 1 °C, 95 ± 5% relative humidity, for a period of three times the

Table 1. Materials, their manufacturer, composition and proportions used.

Sealers	Manufacturer/Composition	Proportion
CS-PG[a]	Powder: tricalcium silicate[b] (46.55%), dicalcium silicate[b] (6.65%), calcium phosphate monobasic[c] (5.00%), calcium hydroxide[d] (3.80%), zirconium oxide[e] (28.50%) and calcium tungstate[e] (9.50%). Liquid: polyethylene glycol 400[e].	1 g/400 µL (powder/liquid)
CS-PGCH[a]	Powder: tricalcium silicate[b], dicalcium silicate[b], calcium phosphate monobasic[c], calcium hydroxide[d], zirconium oxide[e] and calcium tungstate[e]. Liquid: polyethylene glycol 400[e] (400 µL) and chitosan hydrogel[f] (200 µL).	1 g/600 µL (powder/liquid)
TotalFill BC	Brasseler, Savannah, GA, USA. Lot: 15002SP. Valid. 06.2017. Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.	Ready to use
AH Plus	Dentsply DeTrey GmbH, Konstanz, Germany. Lot: 1994151 Valid. 02.2018. Bisphenol A/F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments dibenzyldiamine, aminoadamantane, silicone oil.	1 g/1 g (Paste/paste)

Experimental calcium silicate-base sealer manipulated with polyethylene glycol (CS-PG); Experimental calcium silicate-base sealer manipulated with polyethylene glycol and chitosan hydrogel (CS-PGCH); [a] FOAr-Unesp, Araraquara, SP, Brazil; [b] Mineral Research Processing, Meyzieu, France; [c] Synth, Diadema, SP, Brazil; [d] Merck, Darmstadt, Germany; [e] Sigma-Aldrich, St. Louis, MO, USA; [f] Araraquara School of Pharmaceutical Sciences (FCFAR), UNESP, Araraquara, SP, Brazil.

duration of their setting time), the specimens were removed from their molds, placed in a dehumidifier under vacuum. The mass was measured before and after the specimens were immersed in distilled water with a precision balance (Analítica Adventurer, Model AR2140, Ohaus - Indústria de Balanças Ltda., São Bernardo do Campo, SP, Brazil). The specimens were attached to the closed plastic flasks, containing 7.5 mL of distilled water, with nylon threads and kept in an oven at 37 ± 1 °C for 7 and 30 days. The solubility (mass loss) was expressed as a percentage of the original mass.

2.7 Volumetric change

The experimental sealer with suitable physicochemical properties for clinical use (CS-PG) was selected for additional evaluation of volumetric change, as well as for future studies. The analysis was performed based on a previous study[8], TotalFill BC and AH Plus were used for comparison. The specimens (n = 6) with a diameter of 7.75 mm by 1.50 mm in height were prepared and kept in an oven at 37 ± 1 ºC and relative humidity for 3 times the duration of their setting time. Then, the specimens were scanned by micro-computed tomography SkyScan 1176 (Bruker-MicroCT, Kontich, Belgium). After 7 and 30 days of immersion in distilled water, the samples were placed in a dehumidifier for 24 hours and after this period the specimens were scanned again. The scanning parameters were: 80 kV voltage, 300 μA current, 18 μm voxel size, copper and aluminum (Cu + Al) filter and 360° rotation. The reconstruction of the images was performed using NRecon software (V1.6.10.4; Bruker-MicroCT, Kontich, Belgium). The correction parameter for smoothing, beam hardening, and ring artifacts were defined for each material. The same parameters were used for the same material in the different periods. The reconstructed images were superimposed on the different periods 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 material 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 periods was calculated.

2.8 Statistical analysis

The normality of the data was tested using the Kolmogorov-Smirnov test. Data were submitted to one-way ANOVA and Tukey or Student's t-tests, with 5% significance level.

3. Results and Discussion

Properties of endodontic materials are directly related to the successful root canal treatment[23]. AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is an epoxy resin-based sealer, considered as gold standard due to its physicochemical properties[24]. Therefore, AH Plus, and TotalFill BC, a calcium silicate-based sealer, were evaluated for comparison. The present study assessed two experimental calcium silicate-based sealers manipulated with polyethylene glycol. Both sealers presented proper consistency and promoted an alkaline pH. However, the addition of chitosan impaired some sealer properties, rejecting our null hypothesis.

Polyethylene glycol (PG) as vehicle for calcium hydroxide-based intracanal medication[6] allows diffusion of Ca^{2+} and OH^- ions into root dentin[25]. Polymers such as PG may potentially improve physicochemical properties of silicate materials[26]. PG added to a calcium phosphate-based material (1% ICPC) provided stability, increased viscosity, and allowed cell proliferation[27]. The new bioceramic Bio-C Sealer (Angelus, PR, Brazil) was developed including PG in its composition. Bio-C Sealer has alkalinization ability, suitable flow and radiopacity[8], beside biocompatibility and bioactive potential[7]. For this reason, based on a previous study that showed the setting reaction of this new sealer[8], PG was used as a vehicle for the development of the experimental sealers.

Chitosan was added to the PG in the second experimental sealer (CS-PGCH) aiming to increase antimicrobial activity[17] and bioactive potential[16]. A previous study[16] developed experimental calcium silicate-based sealers manipulated with dicalcium phosphate and chitosan polymer. Although the addition of chitosan did not influence the bioactivity of the materials, an increase in their setting time was observed[16], in agreement with our findings. Since the setting of calcium silicate-based materials depends on moisture[28], the present study used plaster molds to keep this necessary humidity. Our results showed that the experimental sealers, and TotalFill BC presented highest setting times (Table 2) even in the presence of humidity[29,30], as observed in previous studies[29,30]. The properties of setting time, solubility and pH are related[31]. Therefore, the calcium silicate-based sealers had the highest setting time and also showed greater pH and solubility compared to the epoxy resin-based sealer, AH Plus ($p < 0.05$).

After 7 days of immersion in distilled water, the experimental sealers had significantly higher solubility (Table 2) in comparison with AH Plus and TotalFill BC ($p < 0.05$). After 30 days, the experimental sealer with polyethylene glycol presented lower solubility than at 7 days, similar to TotalFill ($p > 0.05$), suggesting a mass stabilization. The results obtained are in accordance with a previous study that observed that experimental sealers containing calcium silicates and calcium phosphate also presented high solubility[32]. iRoot SP (Innovative BioCeramicx, Inc, Burnaby Canada)

Table 2. Setting time, flow, radiopacity, and solubility (mean and standard deviation) observed in the different endodontic sealers.

	AH Plus	TotalFill BC	CS-PG	CS-PGCH
Setting time (min)	384.0 (±0.00)[d]	590.1 (±31.05)[c]	785.0 (±23.45)[b]	870.0 (±0.00)[a]
Flow (mm^2)	407.2 (±114.2)[b]	535.4 (±52.8)[a]	382.8 (±42.4)[b]	250.5 (±46.6)[c]
Flow (mm)	21.41 (±1.14)[b]	24.63 (±0.57)[a]	20.96 (±0.64)[b]	17.46 (±1.39)[c]
Radiopacity (mm Al)	9.22 (±0.44)[a]	5.88 (±0.67)[b]	5.49 (±0.39)[b]	4.51 (±0.50)[c]
Solubility 7 d (% mass loss)	0.04 (±0.48)[d]	7.48 (±0.77)[c]	14.05 (±1.77)[b]	23.84 (±1.51)[a]
Solubility 30 d (% mass loss)	0.31 (±0,33)[c]	10.84 (±3.21)[b]	12.70 (±2.99)[b]	24.44 (±1.79)[a]

[a,b,c] Different letters on the same line indicate statistically significant differences between experimental groups ($p < 0.05$)

has composition similar to the experimental sealers and TotalFill BC, and also shows high solubility[32]. The solubility of calcium silicate-based sealers can be related to the release of OH− and Ca2+ ions and these values are significantly lower in phosphate buffered saline-PBS than in distilled water[33]. CS-PGCH had higher solubility than CS-PG ($p < 0.05$). The larger quantity of vehicle used for the experimental sealer with polyethylene glycol and chitosan hydrogel may be related to its solubility. The addition of chitosan can affect physical and mechanical properties[16]. The solubility of TotalFill BC was in agreement with those observed by Tanomaru-Filho et al.[9] Regarding AH Plus, the low solubility is related to its polymers promoting strong cross-links[32].

The high pH promoted by the calcium silicate-based sealers (Table 3) was previously reported[9,34]. The properties of alkaline pH and calcium ion release may be related to bioactivity, induction of mineralization[35,36], and antimicrobial activity[28]. Thus, some solubility of calcium silicate-based materials may have an effect regarding their biological and antimicrobial activity[31]. In addition, the solubility test is carried out in different conditions from the clinical situation, regarding the contact between the root canal sealer and moisture from tissues in contact to the material[37]. Therefore, the micro-CT evaluation of volumetric stability after 7 and 30 days may complement the solubility test.

The flow of an endodontic sealer is an essential property aiming to fill irregularities in the root canals[38]. AH Plus, TotalFill BC and CS-PG complied with the ISO 6876:2012[19] standard that requires values above 17 mm. Although CS-PGCH has an average flow of 17.46 mm, this material does not comply with the specifications of ISO 6876[19], since some specimens showed flow of less than 17 mm. An additional assessment to complement the conventional test was also performed based on a previous study[20], in which the flow is measured in all area occupied by the sealers (mm^2). The experimental sealer with polyethylene glycol (CS-PG) had flow (mm and mm^2) (Table 2) similar to AH Plus ($p > 0.05$), and greater than CS-PGCH ($p < 0.05$). TotalFill BC had the highest flow rate ($p < 0.05$). Our results regarding the flow of TotalFill BC and AH Plus are in agreement with Tanomaru-Filho et al.[9] The flow ability of bioceramic root canal filling materials are related to their small particle size and appropriate viscosity[39], while the epoxy resin in AH Plus is responsible for its high flow rate[38]. The association of chitosan to the experimental sealer led to a less homogeneous mixture, which can be related to its lower flow (Table 2).

Radiopacity is essential for an endodontic sealer to allows the radiographic analysis of the root canal filling quality[3]. All sealers evaluated had radiopacity above 3 mm Al, in accordance with the recommendation of ISO 6876:2012[19] (Table 2). CS-PG had greater radiopacity than CS-PGCH ($p < 0.05$), and similar to TotalFill BC ($p > 0.05$). These results may be due to the greater amount of liquid present in CS-PGCH. Moreover, the presence of chitosan can affect the physical properties of the materials[16]. Previous studies demonstrated that zirconium oxide promotes proper radiopacity[21] as radiopacifying agent for association with calcium silicate-based cements[40]. Húngaro-Duarte et al.[41] observed that both zirconium oxide and calcium tungstate promoted proper radiopacity, in agreement with our results.

As the experimental sealer with polyethylene glycol and chitosan hydrogel had a longer setting time, less flow and radiopacity, in addition to greater solubility, this sealer was not considered suitable for clinical application. Thus, the CS-PG experimental sealer was selected for the analysis of volumetric change by micro-CT, in comparison with the commercial sealers AH Plus and TotalFill BC. Although the CS-PG had solubility above the maximum recommended by ISO 6876:2012[19], this sealer presented low volumetric change after 7 and 30 days of immersion in distilled water, with the lowest volume loss at 30 days ($p < 0.05$). CS-PG and TotalFill BC had similar volumetric change ($p > 0.05$) after 7 days, and greater values than AH Plus ($p < 0.05$). At 30 days, CS-PG and AH Plus showed similar values ($p > 0.05$), and lower than TotalFill BC ($p < 0.05$) (Table 4). Calcium silicate-based sealers are hydrophilic materials, capable of absorbing water. Therefore, these materials have larger difference in mass after the water has evaporated in the conventional solubility test, which is not appropriate for hydrophilic materials[41]. Previous studies[8,42] observed that calcium silicate-based sealers presented high solubility in the conventional tests. However, different results were observed for the analysis of volumetric change. The volumetric stability of the calcium silicate sealers may be related to the absorption of fluids[43]. Therefore, the volumetric change methodology is important as an additional method to the conventional solubility and dimensional change tests, presenting correlation with the clinical performance of endodontic materials[9].

An important limitation of the current study is that results from *in vitro* studies must be interpreted and extrapolated to clinical situations with caution[44]. Nevertheless, these preliminary results can contribute to further *in vivo* and clinical studies.

Table 3. pH values (mean and standard deviation) observed in the different experimental time intervals (3, 12 and 24 hours, 7, 14 and 21 days).

Periods	AH Plus	TotalFill BC	CS-PG	CS-PGCH	Control
3 h	7.04 (±0.34)^{A,c}	10.70 (±0.25)^{B,a}	10.13 (±0.41)^{AB,b}	10.42 (±0.14)^{AB,ab}	6.15 (±0.29)^{A,d}
12 h	7.42 (±0.38)^{A,c}	11.39 (±0.33)^{A,a}	9.78 (±0.41)^{AB,b}	9.88 (±0.25)^{BC,b}	6.36 (±0.22)^{A,d}
24 h	6.58 (±0.16)^{B,c}	10.37 (±0.20)^{B,a}	9.49 (±0.43)^{B,b}	9.49 (±0.41)^{C,b}	6.50 (±0.17)^{A,c}
7 d	5.73 (±0.29)^{C,d}	10.29 (±0.21)^{B,b}	10.36 (±0.77)^{AB,ab}	10.93 (±0.54)^{A,a}	6.39 (±0.42)^{A,c}
14 d	6.44 (±0.43)^{BC,b}	10.57 (±0.13)^{B,a}	10.58 (±0.47)^{A,a}	10.84 (±0.43)^{A,a}	6.11 (±0.29)^{A,b}
21 d	6.05 (±0.20)^{C,c}	9.50 (±0.93)^{C,b}	10.05 (±0.91)^{AB,b}	10.89 (±0.30)^{A,a}	6.08 (±0.34)^{A,c}

^{ABC}Different capital letter in the same column indicate statistically significant difference among the periods ($p < 0.05$). ^{abcd}Different lower case letters on the same row indicate statistically significant difference among the sealers ($p < 0.05$). Control = distilled water.

Table 4. Volumetric change (7 and 30 days) (mean and standard deviation) observed in the different endodontic sealers.

	AH Plus	TotalFill BC	CS-PG
Volumetric change 7 d, %	1.007 (±0.406)^{A,b}	-1.818 (±0.617)^{A,a}	-2.052 (±1.324)^{A,a}
Volumetric change 30 d, %	-0.328 (±0.100)^{B,b}	-1.875 (±0.898)^{A,a}	-0.347 (± 0.174)^{B,b}

^{AB}Different capital letter in the same column indicate statistically significant difference between the periods ($p < 0.05$). ^{ab}Different lower case letters on the same row indicate statistically significant difference among the sealers ($p < 0.05$). Negative values indicate volume loss.

4. Conclusions

Calcium silicate-based experimental endodontic sealer with polyethylene glycol presented proper setting time, radiopacity, flow and pH, besides low volumetric change, showing better results than CS-PGCH, and potential for clinical application.

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