



Physicochemical Properties and Bioactive Potential of a New Epoxy Resin-based Root Canal Sealer

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The aim of this study was to evaluate physicochemical properties and bioactive potential of Sealer Plus, in comparison with MTA Fillapex, Sealapex and AH Plus. Setting time, flow, and radiopacity were evaluated based on ISO 6876 Standard. Flow was also assessed in area (mm²). The solubility and volumetric change of the sealers were evaluated after 7 and 30 days of immersion in distilled water. Solubility was evaluated by the difference in mass of materials before and after immersion. Volumetric change was evaluated by using microcomputed tomography (micro-CT). The bioactive potential was observed by Scanning Electron Microscopy (SEM) after immersion in PBS. Data were compared using ANOVA and Tukey tests ($\alpha=0.05$). Sealer Plus presented the shortest setting time (196 min.) and Sealapex the longest (912 min.) ($p<0.05$). AH Plus showed the highest radiopacity (9.5 mm Al) and MTA Fillapex the lowest (2.7 mm Al) ($p<0.05$). All the sealers presented flow in accordance with ISO 6876/2012 (>17 mm). Sealer Plus showed low solubility and volumetric change ($<1\%$), and MTA Fillapex showed the highest solubility ($>25\%$), and volumetric change ($>4\%$) after all time intervals ($p<0.05$). MTA Fillapex was the only sealer that showed bioactive potential. In conclusion, Sealer Plus presented proper physicochemical properties. However, this sealer did not present a bioactive potential.

Key Words: endodontics, physicochemical properties, root canal filling material, x-ray microtomography.

Introduction

The development of sealers with proper physicochemical and biological properties is important for successful endodontic treatment (1). Moreover, root canal sealers should be bioactive stimulating repair of the periapical tissues (2).

Root canal filling materials are investigated by using standardized tests to evaluate setting time, flow, solubility, and radiopacity (3). The setting time should be long enough to allow the root canal filling (3). A proper flow allows sealer to penetrate into the irregularities of root canal system (4). A low solubility prevents change in structure or integrity of the sealer (5). The radiopacity allows radiographic evaluation of root canal filling (4). Currently, micro-computed tomography (micro-CT) have been proposed to evaluate physical properties of endodontic materials (6-8). Micro-CT is a non-destructive tool, which presents the ability to evaluate materials three-dimensionally over time (7,8).

Endodontic sealers are divided according to their main components, such as resin, calcium oxide, and calcium silicate (9). The properties of these sealers are directly related to their composition (1). The presence of calcium oxide in the composition of endodontic materials has been suggested due to their ability to induce the formation of mineralized tissues, favoring the repair (9). Mineral Trioxide

Aggregate (MTA) is widely used in Endodontics for vital pulp therapy, regenerative endodontic procedures, apical barrier in teeth with open apices, perforation repair, and root-end filling (10). The application of MTA promotes reduced inflammation and greater deposition of cementum (11). Root canal sealers based on salicylate resin combined with MTA or calcium oxide are available on the market. However, both materials have demonstrated high solubility (3,12). Epoxy resin-based sealer is considered the gold-standard for physicochemical properties (13), in spite of not showing bioactive potential (2). Recently, a new epoxy resin-based sealer was developed with the presence of calcium hydroxide in its composition. There are few studies evaluating this sealer, which showed proper biological (1) and physicochemical (4) properties.

The aim of this study was to evaluate setting time, solubility, radiopacity, flow, volumetric stability, and bioactive potential of root canal filling materials. Therefore, this study was designed to evaluate the experimental hypothesis that endodontic sealer compositions affect the physicochemical properties of these sealers.

Material and Methods

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

Setting Time

The setting time test was conducted in accordance with ISO Standard 6876 (14). After manipulation, the sealer was placed in metal rings measuring 10 mm in diameter and 1 mm high (n=8). A Gilmore needle with mass of 100 ± 0.5 g and diameter of 2.0 ± 0.1 mm was used, supported on the sealer surface. The sealers were kept in an oven at 37 °C and 95% humidity. The setting time was considered as the time when the marks of needle could not be observed on the sealer surface.

Radiopacity

The radiopacity test was performed based on ISO Standard 6876 (14). Stainless steel rings measuring 10 mm in diameter and 1 mm high were used to fabricate 6 test specimens of each sealer. The specimens and an aluminum scale were placed on an occlusal film (Insight; Kodak Comp, Rochester, NY, USA) to take the radiograph (X-ray appliance -X GE 1000; General Electric, Milwaukee, WI, USA). The parameters used were 60 kV, 7 mA, 0.32 pulses per second and focal distance of 33 cm. The exposed films were processed, digitized, and evaluated by using Image J for Windows software, to determine the radiopacity equivalence of the sealers in millimeters of aluminum (mm Al).

Flow

The flow test was conducted in accordance with ISO Standard 6876 (14). After manipulation, 0.05 mL of the sealer was placed in the center of a glass plate by using a graduated syringe (n=6). At 180 ± 5 s 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 min. After this period, the maximum and minimum diameters of the sealer on the glass plate were measured, using a digital pachymeter (Mitutoyo MTI Corp., Huntersville, NC). When a difference

of less than 1 mm between the diameters was observed, the mean value was recorded. A second evaluation was made by photographing the sealer on the plate alongside a millimeter ruler. The images obtained were evaluated using the Image Tool 3.0 software (UTHSCSA, San Antonio, TX) to obtain the area of flow of the sealer expressed in mm², according to Tanomaru-Filho et al. (15).

Solubility

Based on a previous study (5), circular plastic molds measuring 1.5 mm high and 7.75 mm in diameter were placed on a glass plate covered with cellophane film. These molds were filled with each sealer (n=6). A nylon thread was embedded in the fresh sealer mixture, and another glass plate covered by cellophane was placed over the mold. After 3 times the duration of their setting time, the test specimens were removed from the molds and weighed in an analytical balance with accuracy of ± 0.0001 g (AR2140, Toledo do Brasil Indústria de Balanças Ltda., São Bernardo do Campo, SP, Brazil), and the mean reading was recorded. The samples were placed in plastic receptacles with lids containing 7.5 mL of distilled and deionized water, suspended by nylon threads attached to the containers. The receptacles remained in the oven at 37 °C for 7 days. After this period, the test specimens were removed from the distilled water, dried and placed in a dehumidifying chamber. The mass was measured before and after immersion of the samples in distilled water, and every 24 h thereafter, until attaining mass stabilization. New samples were prepared and kept immersed in distilled water for 30 days. The mass loss was expressed as a percentage of the original mass.

Volumetric Change

Volumetric change of the sealers was analyzed based on previous studies (7,8). Transparent acrylic resin-based models were fabricated using metal molds with cavities

Table 1. Endodontic sealers, their manufacturers and composition

Sealer	Manufacturer	Composition
MTA Fillapex	Angelus Dental Solutions, Londrina, PR, Brazil	<i>Base paste</i> : salicylate resin, natural resin, calcium tungstate, nanoparticulated silica, pigments; <i>Catalyst paste</i> : diluting resin, mineral trioxide aggregate, nanoparticulated silica, pigments
Sealapex	SybronEndo - Sybron Dental Specialties, Glendora, CA, USA	<i>Base paste</i> : sulphonamide resin, N-ethyl toluene, silicon dioxide, zinc oxide, calcium oxide; <i>Catalyst paste</i> : isobutyl salicylate resin, silicon dioxide, bismuth trioxide, titanium dioxide, pigments
Sealer Plus	MK Life - Medical and Dental Products Brasil, Porto Alegre, RS, Brazil.	<i>Base paste</i> : Bisphenol A-co-epichlorohydrin, Bisphenol F epoxy resin, zirconium oxide, silicone and siloxanes, iron oxide (pigment), calcium hydroxide; <i>Catalyst paste</i> : Hexamethylenetetramine, zirconium oxide, silicone and siloxanes, calcium hydroxide, calcium tungstate
AH Plus	DentsplyDeTrey, Konstanz, Alemanha	<i>Paste A</i> : Bisphenol epoxy resin-A, Bisphenol epoxy resin-F, calcium tungstate, zirconium oxide, silica, iron oxide pigments; <i>Paste B</i> : Dibenzylidiamine, aminodiamantana, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil.

measuring 3 mm deep and 5 mm in diameter (n=6). The cavities were filled with each sealer and were kept in an oven at 37 °C and relative humidity for 3 times the duration of their setting time. Scanning was performed by micro-CT imaging (SkyScan 1176; Bruker-microCT, Kontich, Belgium) and completed using 50 kV X-ray tube voltages and 500 µA anode current; aluminum filter of 0.5; isotropic voxel of 18 µm; and an evolution cycle of 360°. The samples were scanned after setting and after 7 and 30 days of immersion of the specimens in distilled water. The images were reconstructed using NRecon software (V1.6.10.4; Bruker-MicroCT). The correction parameters for smoothing, beam hardening and ring artefacts were defined for each material (the parameters for AH Plus were 2 for smoothing, 60 for beam hardening correction and 2 for ring artefacts correction, for Sealapex were 2 for smoothing, 55 for beam hardening and 2 for ring correction, for Sealer Plus were 1 for smoothing, 50 for beam hardening and 1 for ring and for MTA Fillapex, 1, 40 and 1, respectively). The same parameters were used for the same materials in the different periods. The reconstructed images were superposed at the different experimental time intervals using the Data Viewer software (V1.5.2.4; Bruker-MicroCT, Kontich). The images were used for quantitative analysis of the samples, allowing the total volume of sealer to be calculated in mm³ by CTAn software (V1.15.4.0; Bruker-MicroCT). The volumetric change was calculated at each time interval.

Bioactive Potential

The bioactivity test was performed based on a previous study (16). The sealers were manipulated, inserted into cylindrical molds measuring 1 mm high x 7.5 mm in diameter and remained in an oven at 37°C and 95% humidity until the complete setting. Subsequently, the samples were removed from the molds, and immersed in PBS solution (for 500 mL of distilled water; anhydrous NaCl – 90 g, Na₂HPO₄ – 13.65g; Na₂H₂PO₂H₂O – 2.42 g), at 37°C for 30 days. After this time interval, the samples were removed from the solution, and dried in a dehumidifier for 7 days, when their surfaces were metalized with carbon and analyzed by scanning electron microscopy (JSM-6610LV Scanning Electron Microscope, JEOL, Tokyo, Japan), in secondary electron (SE) mode with the purpose of identifying the mineral deposition on the sealer surfaces.

Statistical Analysis

The results obtained for all the tests were submitted to a normality test, and then to the

parametric ANOVA statistical test and the Tukey multiple comparison test, with 5% significance level.

Results

Sealer Plus presented the shortest setting time, followed by AH Plus, MTA Fillapex and Sealapex (p<0.05). AH Plus showed the highest radiopacity, followed by Sealapex and Sealer Plus (p<0.05). MTA Fillapex had the lowest radiopacity (p<0.05). MTA Fillapex showed the highest flow, followed by AH Plus (p<0.05). Sealapex and Sealer Plus presented the lowest flow, which similar values (p>0.05). The results are represented in Table 2.

The results for solubility and volumetric change are shown in Table 3. MTA Fillapex presented the highest solubility and volumetric change (volume loss) in both periods, followed by Sealapex (p<0.05). Sealer Plus and AH Plus showed the lowest solubility and volumetric change (p>0.05).

The external surface of the sealers in contact with PBS for 30 days was evaluated by scanning electron microscopy (SEM). The micrographs of the samples after the bioactive potential assay are represented in Figure 1. Sealapex, Sealer Plus, and AH Plus presented a similar microstructure. Except for MTA Fillapex, during the qualitative analysis, it was not possible to observe any mineral deposition on sealers surface (Fig. 1B, C, D). On the other hand, Figure 1A shows the

Table 2. Means and standard deviation of setting time, radiopacity, and flow of the root canal sealers

Sealer	Setting Time (min)	Radiopacity	Flow	
		(mm Al)	(mm)	(mm ²)
MTA Fillapex	637 ± 23 ^b	2.70 ± 0.30 ^d	28.78 ± 0.32 ^a	683.80 ± 32.11 ^a
Sealapex	912 ± 14 ^a	7.80 ± 0.60 ^b	19.14 ± 0.71 ^c	368.10 ± 10.92 ^c
Sealer Plus	196 ± 14 ^d	4.00 ± 0.90 ^c	18.95 ± 0.74 ^c	381.70 ± 73.77 ^c
AH Plus	497 ± 19 ^c	9.50 ± 0.30 ^a	21.94 ± 0.74 ^b	516.10 ± 67.53 ^b

Different letters in the same column indicate statistically significant difference (p<0.05).

Table 3. Means and standard deviation of solubility and volumetric change of the root canal sealers at the different experimental periods

Sealer	Solubility (mass loss) (%)		Volumetric change (%)	
	7 days	30 days	7 days	30 days
MTA Fillapex	25.69 ± 6.77 ^a	30.19 ± 3.08 ^a	-4.03 ± 1.09 ^a	-6.36 ± 1.81 ^a
Sealapex	12.83 ± 1.25 ^b	12.43 ± 2.37 ^b	-1.48 ± 0.34 ^b	-1.50 ± 0.40 ^b
Sealer Plus	0.45 ± 0.20 ^c	0.95 ± 0.21 ^c	0.46 ± 0.28 ^c	0.84 ± 0.54 ^c
AH Plus	0.20 ± 0.08 ^c	0.21 ± 0.07 ^c	0.27 ± 1.13 ^c	-0.52 ± 0.09 ^{bc}

Different letters in the same column showed statistically significant difference (p<0.05). Negative values in the volumetric change test indicate volume loss.

structures that suggest the presence of calcium phosphate deposits on the surface of MTA Fillapex, suggesting its bioactive potential.

Discussion

ISO 6876/2012 Standard (14) recommend values in accordance with the manufacturers' indication for sealers with setting time between 30 min to 72 h. The setting time of sealers depends on their components (17). In the present study, Sealer Plus showed the shortest setting time, followed by AH Plus, which are epoxy resin-based sealers. The addition of calcium hydroxide in Sealer Plus may justify its shorter setting time, which could be related to the hydration of the sealer (18). The values observed in this study are in accordance with Vertuan et al. (4). MTA Fillapex and Sealapex showed longer setting time, as observed by Camilleri (19) and Viapiana et al. (3). This long setting time to MTA Fillapex can be related to salicylate resin. Salicylate resin-based endodontic sealers may not set in a dry environment (17).

Setting time and solubility may be related, whereas a long setting time could favor solubility (5). Our study evaluated the solubility of the sealers based on a previous study that proposed samples with smaller dimensions without affecting the accuracy of the test (5). This methodology has been used to assess endodontic materials with different compositions (3,7,8). MTA Fillapex presented the highest solubility in both time intervals (7 and 30 days) followed by Sealapex. Both materials are salicylate resin-based sealers. The epoxy resin-based sealers (AH Plus and Sealer Plus) showed lower solubility, which may be related to its polymers with strong cross-links (12). Our results corroborate Silva et al. (20), who evaluated the dissolution of MTA Fillapex and AH Plus and observed a solubility of over 20% for MTA Fillapex after 28 days, while AH Plus maintained values below 1%. Viapiana et al. (3) also observed that Sealapex and MTA Fillapex had high solubility in water, and the authors attributed the high solubility of Sealapex to its setting reaction, resulting in a fragile matrix (12).

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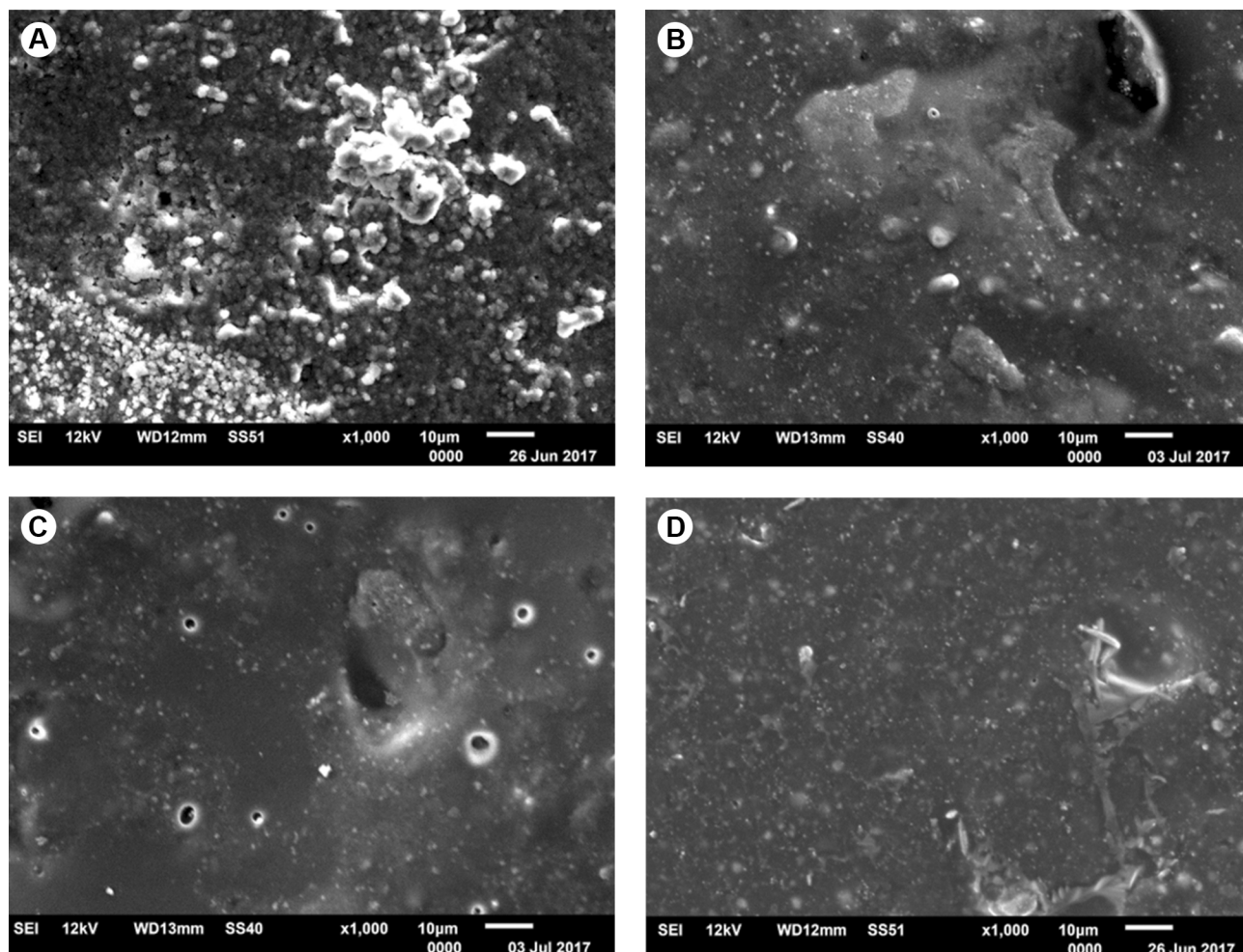


Figure 1. Representative images of each sealer after SEM analysis. A: MTA Fillapex, B: Sealapex, C: Sealer Plus; and D: AH Plus.

Although solubility of sealers may not allow proper sealing of root canal systems (4,5), the alkalization ability is closely connected to the long setting time and solubility of materials (4). So, the highest solubility of MTA Fillapex probably resulted Ca^{2+} ion release (12). In the present study, the bioactive potential of the root canal sealers was evaluated by SEM after 30 days of immersion in PBS. The images obtained showed that only MTA Fillapex induced a mineral deposition on its surface, which may be related to the capacity of MTA for inducing mineralized tissue (21). Bioactive potential was not observed for the epoxy resin-based sealers, probably due to the lack of ionized calcium ions (2). Even with the presence of calcium hydroxide in their composition, Sealapex and Sealer Plus did not show bioactive potential. Our results corroborate Vertuan et al. (4), who observed that Sealer Plus was not able to promote alkalizing. The authors justify this finding as a result of the epoxy resin in the composition of the sealer, and the low solubility of Sealer Plus.

The conventional test performed to evaluate the solubility of endodontic sealers present some limitations, since the sealers can have water uptake or disintegration during the storage (7). These conditions cannot be observed only comparing the initial and final mass of the samples, leading to the search for new methodologies (8). Micro-CT is widely applied due its ability to allows three-dimensional evaluation (22). The volumetric change for endodontic materials measured by micro-CT after immersion in distilled water for time intervals of 7 and 30 days allows three-dimensional evaluation, complementing the conventional solubility test (6,8).

When Silva et al. (6) investigated the volumetric change for AH Plus and MTA Fillapex in human teeth after immersion in PBS, they observed no statistical differences between the sealers, in disagreement with our results. This divergence could be related to the methodology and immersion solution used. Our results showed a volumetric loss for both MTA Fillapex and Sealapex, which could be explained by the high solubility of these sealers. Although Sealer Plus presented a mass loss in the solubility test, the sealer showed increase in volume, which suggested expansion after immersion in water. This is the first study to evaluate the volumetric change of Sealer Plus. A previous study evaluated the physicochemical properties of AH Plus after incorporation of calcium hydroxide, and observed an increase in the sealer expansion. The authors attributed the expansion to the hygroscopic effect of calcium hydroxide, which might have favored a higher water sorption and led to expansion of the sealer (23). This statement can justify the volume increase of Sealer Plus in the current study, since the volumetric changes do not depend on the solubility only, but also is related to dimensional changes

such as contraction or expansion (6,8). Thus, volumetric change represents important information suggesting the clinical perspective of the sealer after root canal filling (7).

Radiopacity of the sealers allows its differentiation in relation to the adjacent anatomic structures (24). The ISO 6876/2012 Standard (14) recommends the minimum radiopacity value equivalent to 3 mm Al. MTA Fillapex demonstrated a radiopacity lower than the minimum recommended. Although proper radiopacity has been reported for MTA Fillapex (17), the present study evaluated the new version of the sealer that present the radiopacifying agent calcium tungstate as a substitute for bismuth oxide (25). A justification for the low values for MTA Fillapex may be related to lower radiopacifying potential of calcium tungstate (24). Although Sealer Plus showed radiopacity lower than AH Plus and Sealapex sealers, this sealer had a radiopacity in accordance with the ISO Standard, as observed in a previous study (4). Sealer Plus contains two chemical components responsible for its radiopacity, zirconium oxide and calcium tungstate.

The flow ability of root canal sealers is an important property to fill irregular areas of the root canal system (23). In this study, two methods of analysis were used to evaluate flow ability of the sealers. The first method was in accordance with the ISO 6876/2012 (14), using the diameter of the sealer after test, in millimeters. In order to complement the conventional test, which does not evaluate all area occupied by the sealers, an additional analysis was performed by means of the area (mm^2), as proposed by Tanomaru-Filho et al. (15). All the sealers showed flow in accordance with the ISO specification, which recommends a minimum flow of 17 mm, corroborating with previous studies (3,4). Sealer Plus and Sealapex had lower values than AH Plus, probably because the calcium hydroxide decreases the flow of resin-based sealers (23). MTA Fillapex presents a film thickness less than $50 \mu\text{m}$ (19), a property that could justify its highest flow values.

Sealer Plus is a recent sealer and few studies evaluated its properties, leading to the need of more investigations regarding the behavior of this sealer in a clinical environment. Sealer Plus shows proper physicochemical properties, such as flow, radiopacity, setting time, solubility, and volumetric stability. However, this sealer does not show bioactive potential.

Resumo

O objetivo deste estudo foi avaliar as propriedades físico-químicas e o potencial bioativo de Sealer Plus, em comparação com MTA Fillapex, Sealapex e AH Plus. Tempo de presa, escoamento e radiopacidade foram avaliados com base nas normas ISO 6876. O escoamento foi também avaliado em área (mm^2). A solubilidade e a alteração volumétrica dos cimentos foram avaliadas após 7 e 30 dias de imersão em água destilada. A solubilidade foi analisada pela diferença entre as massas dos cimentos

antes e após imersão. A alteração volumétrica foi avaliada por meio de microtomografia computadorizada (micro-CT). O potencial bioativo dos cimentos foi observado por Microscopia Eletrônica de Varredura (MEV) após imersão em PBS. Os dados foram comparados usando os testes ANOVA e Tukey ($\alpha=0.05$). Sealer Plus apresentou o tempo de presa mais curto (196 min.) e Sealapex o mais longo (912 min.) ($p<0.05$). AH Plus mostrou a radiopacidade mais alta (9.5 mm Al) e MTA Fillapex a mais baixa (2.7 mm Al) ($p<0.05$). Todos os cimentos tiveram escoamento de acordo com as normas ISO 6876/2012 (>17 mm). Sealer Plus mostrou baixos valores de solubilidade e alteração volumétrica ($<1\%$) e MTA Fillapex teve os valores mais altos de solubilidade ($>25\%$) e alteração volumétrica ($>4\%$) em todos os períodos avaliados ($p<0.05$). MTA Fillapex foi o único cimento que mostrou potencial bioativo. Como conclusão, Sealer Plus apresentou propriedades físico-químicas adequadas, entretanto, não apresentou potencial bioativo.

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Received May 21, 2019

Accepted July 29, 2019