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Evaluation of Selective Physicochemical and Biological Properties of Different Root Canal Sealers

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ABSTRACT

Introduction: This *in vitro* study aimed to evaluate the chemical composition, water solubility, radiopacity, pH, electrical conductivity and cytotoxicity of four different root canal sealers. **Methods and Materials**: Four materials were tested including an epoxy resin-based sealer (AH-Plus), a calcium silicate-based sealer (MTA Fillapex), a calcium hydroxide-based sealer (Sealapex) and a zinc-oxide-eugenol-based sealer (Endofill). The materials were submitted to energy-dispersive x-ray microanalysis for elemental chemical composition. Solubility and radiopacity were evaluated according to ANSI/ADA. The pH and electrical conductivity were measured at different periods of time. L929 immortalized mouse fibroblast line were used for cytotoxicity evaluation. Statistical analyses were carried out using the ANOVA and Tukey's test. **Results:** The main elements were found to be silicon and calcium in MTA Fillapex, calcium and bismuth in Sealapex, zirconium and tungsten in AH-Plus and zinc and bismuth in Endofill. Sealapex had the highest value for solubility ($P<0.05$), AH-Plus showed the highest radiopacity value ($P<0.05$) while MTA Fillapex had the highest pH and electrical conductivity values ($P<0.05$). AH-Plus showed the highest rate of cell viability ($P<0.05$). **Conclusion:** Based on the results of this *in vitro* study, it was possible to conclude that Endofill and Sealpex did not meet the requirements for water solubility. The tested sealers were alkaline and showed radiopacity in accordance with ANSI/ADA standards. AH-Plus showed to be less cytotoxic than other tested root canal sealers.

Keywords: Biological Assay; Endodontics; Root Canal Filling Materials; Root Canal Obturation

Introduction

Successfull root canal treatment (RCT) requires a combination of mechanical instrumentation and chemical debridement, followed by filling with biocompatible materials [1, 2]. A hermetic three-dimensional obturation is one of the main goals of RCT and involves the association of gutta-percha and a root canal sealer (RCS) [1, 3]. The contribution of RCS to the success of RCT is related to an airtight seal, which may avoid bacterial leakage and consequently prevent oral pathogens from colonizing and re-infecting root canal space and periapical tissues [3-5].

A RCS should present low solubility, not irritate periapical tissues and ought to reduce the formation of gaps between the canal and the obturation materials [6, 7]. Besides, they should present adequate radiopacity, so that they could be distinguished from the surrounding anatomical structures and reveal empty spaces and inappropriate contours [8, 9].

A high alkaline pH is associated with a biocompatible condition which provides cell adhesion and differentiation, stimulating mineralized tissue formation [10, 11]. The biocompatibility of a RCS is affected by its composition, microstructure and surface characteristics [7, 11-13].

Eugenol-based sealers, due to their satisfactory physicochemical properties and adhesion, are still widely employed in Endodontics [14]. Zinc-oxide-eugenol-based sealers (*e.g.* Endofill) have a composition based on a silicone elastomer (monomer and silicone based catalyst) and a subnitrate bismuth filler [15].

Calcium hydroxide-based RCSs (*e.g.* Sealapex) have been used because of their better biocompatibility [16] and improved seal of root canal system [9, 17].

Epoxy-resin-based sealers (*e.g.* AH-Plus) are used as a standard reference; owing to their long-term dimensional stability, adherence to root dentine, antimicrobial activity, adequate biological properties and low solubility [5].

RCSs based on mineral trioxide aggregate (MTA) (*e.g.* MTA Fillapex) has been reported to be biocompatible, stimulate mineralization and enhance apatite-like crystalline deposits along the apical- and middle-thirds of root canal walls [10, 16, 18]. These sealers combine the physicochemical properties of a sealer [19, 20] with the biological properties of MTA [11, 21].

Since there are many RCSs available on the market, it is important to perform periodical screening using independent studies to obtain scientific evidence and promote evidence-based dentistry. In literature, there are not many studies which link and compare physicochemical and biological properties at same time. The aim of this study was to compare selected physicochemical properties and the cytotoxicity of an epoxy resin-based sealer (AH-Plus), a calcium silicate-based sealer (MTA Fillapex), a calcium hydroxide-based sealer (Sealapex) and a zinc-oxide-eugenol-based sealer (Endofill). The null hypothesis was that different RCSs would show similar performance.

Table 1. Compositions of the materials used in present study.

Tested Material	Manufacturer	Composition	Batch Number #
AH-Plus	Dentsply, De Trey Gmbh, Konstanz, Germany	Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments Paste B: dibenzyldiamine, aminoadamantane, tricyclodecanediamine, calcium tungstate, zirconium oxide, silica, silicone oil	1108000936
MTA Fillapex	Angelus, Londrina, PR, Brazil	Paste A: methyl salicylate, butylene glycol, colophony, bismuth trioxide, fumed silicon dioxid Paste B: fumed silicon dioxide, titanium dioxide, tricalcium silicate, dicalcium silicate, calcium oxide, tricalcium aluminate, pentaerythritol, rosinate, p – toluenesolfonamide	21787
Sealapex	SybronEndo, Romulus, MI, USA	Base: N-ethyl toluene solfanamide resin, silicon dioxide, zinc oxide, calcium oxide Catalyst: isobutyl salicylate resin, silicon dioxide bismuth trioxide, titanium dioxide pigment	1-1267
Endofill	Dentsply, Petrópolis, RJ, Brazil	Zinc oxide, hydrogenated resin, bismuth subcarbonate, barium sulfate, sodium borate, eugenol and oil of sweet almonds	556419D

Table 2. Elemental composition of root canal sealers determinated by Energy-dispersive X-ray microanalysis

Element	MTA Fillapex		Sealapex		AH-Plus		Endofill	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
C	17.79	34.01	12.56	29.84	34.98	67.22	14.81	41.53
O	26.20	37.60	18.00	32.08	14.36	20.72	14.03	29.54
Al	0.45	0.38	0.46	0.49	-	-	-	-
Si	16.79	13.72	3.93	3.99	-	-	-	-
S	4.66	3.33	3.35	2.98	-	-	-	-
Ca	14.87	8.52	32.42	23.08	3.40	1.96	-	-
Ti	0.88	0.42	4.16	2.48	-	-	-	-
Zn	-	-	5.45	2.38	-	-	47.16	24.30
Bi	18.37	2.02	19.66	2.68	-	-	14.94	2.41
Cl	-	-	-	-	0.37	0.24	-	-
Zr	-	-	-	-	31.16	7.88	-	-
W	-	-	-	-	15.72	1.97	-	-
Ba	-	-	-	-	-	-	9.06	2.22

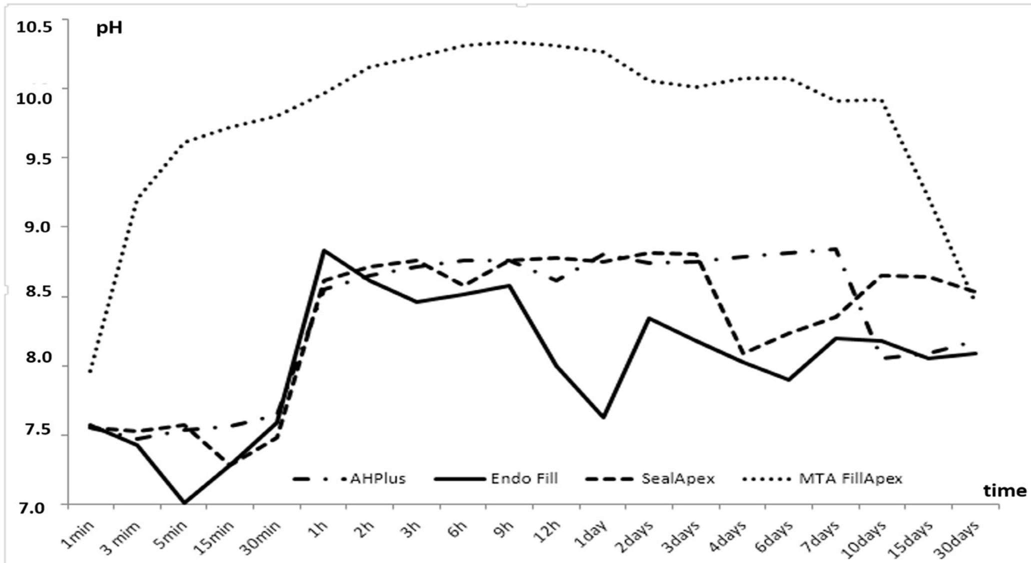

Figure 1. pH evaluation according to different periods of time

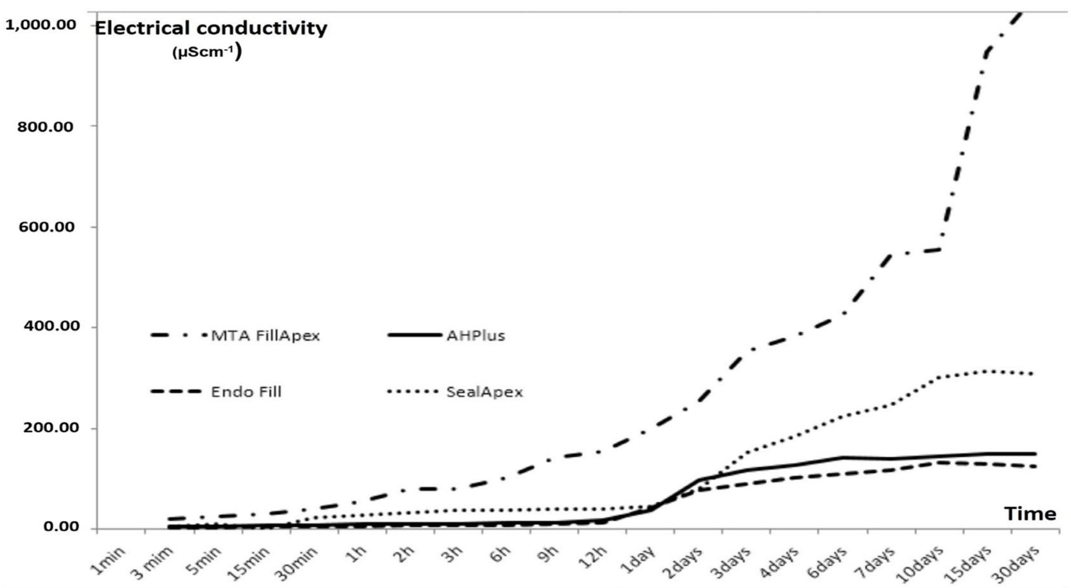

Figure 2. Electrical conductivity (µS/cm-1) evaluation according to different time periods

Material and Methods

The four tested RCSs used in this *in vitro* study are described in Table 1. These sealers were handled by a single user and mixed according to manufacturer's instructions.

Energy-dispersive X-ray analysis (EDX)

Three cylindrical teflon molds (3.0×4.0 mm) were filled with freshly mixed RCSs. The molds were supported by a glass plate covered with a mylar strip and kept in a chamber at 37±1°C and 95±5% relative humidity for 24 h. Afterwards, the samples were sprinkled on carbon double-sided tape over a metallic stub, critical-point dried and sputter-coated with gold-palladium (Bal-Tec AG, Balzers, Liechtenstein, Germany) at 20 mA. EDX was performed using the NSS Spectral Analysis System 2.3 (Thermo Fischer Scientific, San Jose, CA, USA) to determine the chemical composition of the materials. One EDX spectrum was obtained from the central region of each specimen under the following conditions: 25 kV accelerating voltage, 110 µA beam current, 10-6 Torr pressure (high-vacuum), 130×130 µm area of analysis at 1.000× magnification, 100 sec acquisition time and 30-35% detector dead time. The elemental analysis [weight% (wt %) and atomic% (at %)] of samples was conducted using nonstandard analysis mode and the Phi-Rho-Z (Proza) correction method.

Analysis of selective properties

The water solubility test was determined in accordance with ANSI/ADA specification number 57 [22]. For radiopacity evaluation, five acrylic plates (2.2 cm×4.5 cm×1 mm) with 4 holes measuring 1 mm in depth and 5 mm in internal diameter were fulfilled with the tested sealers. Each acrylic plate containing the sealers, was positioned together with another acrylic plate (1.3 cm × 4.5 cm × 1 mm), which contained a graduated aluminum stepwedge varying from 1 to 10 mm in thickness, and uniform steps of 1 mm each. The Digora™ system (Soredex Orion Corporation, Helsink, Finland) was used and, after being exposed, it was inserted into the laser optical reader of Digora™ for Windows 5.1 software. The same phosphorus plate was used for all exposures.

For pH analysis, the samples were prepared like the obtained samples for the solubility test. Distilled water pH measurements were taken with a pH meter (Corning Inc, New York, USA) in different periods of time: 1, 3, 5, 15, 30 min; 1, 2, 3, 4, 6, 9, 12, 24, 48, 72 h; 4, 6, 7, 15 and 30 days after spatulation. During the experiment, pH was analysed for each sample in the same plastic recipient without liquid substitution. pH was measured five times for each sealer.

After pH analysis, the sample was retained in the plastic recipient and at the same time periods, the electrical conductivity of the solution was measured. All 5 samples of each sealer were analysed with a condutivimeter (Marconi Equip. Ltda, Piracicaba, Brazil). The device was calibrated according to a calibration curve obtained from a solution of 1.412 μScm^{-1}.

Cytotoxicity analysis

Mouse fibroblasts of the L929 immortalized cell line were cultured in cell culture medium DMEM (Dulbecco Modified Eagle Medium) and incubated at 37ºC in a controlled atmosphere of 5% CO_2 in air until sub confluence was achieved. DMEM was supplemented with 10% fetal bovine serum (FBS, PAA, Pasching, Austria), 2% LM glutamine, penicillin (100 U/mL) and streptomycin (100 mg/mL).

For cell viability assay (ISO 10993-5) [23], mouse fibroblasts L929 (2×10^4/well) were maintained in DMEM in 96-well plates for 24h. The 3M (4,5 MdimethylthiazolM2Myl) M2-5Mdiphenyltetrazolium bromide (MTT) assay (Sigma Chemical Company, St. Louis, MO, USA) was used to assess cell metabolic function *via* colorimetric assay. To evaluate the cytotoxicity of the RCSs, they were poured into sterile circular moulds (5 mm in diameter and 1mm deep) and covered with a mylar strip (*n*=6). Each specimen was previously incubated in 1mL of DMEM at 37ºC and at pH of 7.2 for 24 h under static conditions to obtain the eluates.

The positive control used was the untreated group (only fibroblast cells in DMEM, without eluate addition). The eluates from the specimens were applied in each well of 96-well plates; previously prepared with mouse fibroblasts. The cytotoxicity produced, by each different group, was assessed with 24h cell exposure time. After removing the eluates, 180μL of DMEM was added to 20μL of MTT solution and placed in each well. After 4h of incubation at 37ºC in darkness, the blue formazan precipitate was extracted from the mitochondria using 200μL/well of dimethyl sulfoxide on a shaker at 150rpm for 5min. The absorption was determined using a spectrophotometer at a wavelength of 540nm.

Statistical analysis

For each test, the data were statistically analysed by one-way ANOVA and Tukey's post-hoc test at 5% level of significance. When sample distribution was non-normal, nonparametric analysis of variance was performed using Kruskal-Wallis test (α=0.05). The tests were performed with the SPSS for Windows statistical software; version 12.0.1 (SPSS Inc., Chicago, IL, USA).

Results

The quantitative results of the main components of the tested sealers are shown in Table 2. The EDX analysis revealed that MTA Fillapex was mostly composed of O (26.20 wt%), Bi (18.37 wt%), C (17.79 wt%), Si (16.79 wt%) and Ca (14.87 wt%), Sealapex of Ca (32.42 wt%), Bi (19.66 wt%), O (18.00 wt%) and C (12.56 wt%), AH-Plus of C (34.98 wt%), Zr (31.16 wt%), W (15.72 wt%) and O (14.36 wt%) and Endofill of Zn (47.16 wt%), Bi (14.94 wt%), C (14.81 wt%) and O (14.03 wt%).

Table 3. Physicochemical properties of the tested materials [mean (SD)]

Properties	Root canal sealers			
	AH-Plus	Endofill	MTA Fillapex	Sealapex
Solubility (%)	0.29 (0.04)^a	3.81 (0.37)^c	2.88 (0.24)^b	5.45 (0.46)^d
Radiopacity (mm Al)	1.64 (0.07)^a	1.46 (0.06)^b	1.45 (1.45)^b	1.48 (0.008)^b
pH	8.34 (0.52)^a	8.02 (0.48)^a	9.78 (0.63)^b	8.29 (0.60)^a
Electrical conductivity (μScm^{-1})	63.46 (62.61)^a	52.07 (52.66)^a	286.60 (303.54)^a	117.20 (113.87)^a

**Different superscript letters in same line represents statistically significant difference (P<0.05)*

Table 3 shows the mean values and standard deviations of selective physicochemical properties of the tested RCSs. Sealapex had the highest mean value for the solubility whilst AH-Plus had the lowest one (*P* <0.05). Although all the RCSs were in accordance with ANSI/ADA number 57 (above step 3=1.17 ± 0.10 mm Al), AH-Plus (1.64±0.07) presented the highest radiopacity mean value amongst the tested sealers (*P* <0.05).

MTA Fillapex presented the highest mean value for pH (*P* <0.05). There was no significant difference in the mean values for the pH reading of AH-Plus, Endofill and Sealapex during all the time periods evaluated (Figure 1). From day 1 to the end of the period of the tests, MTA Fillapex showed the highest values of electrical conductivity (*P* <0.05) (Figure 2).

Table 4 shows the percentage of the assessed cell viability after 24h. The untreated group (cell control without eluate resin) was considered 100%. AH-Plus was statistically different from all groups (*P*<0.05), and showed cell viability of 66%.

Discussion

Before a material is used for clinical purposes, different aspects and perspectives of the material should be taken into consideration. Therefore, characteristics of a root canal sealer, such as tissue tolerance, physicochemical properties, and antimicrobial and clinical characteristics, ought to be initially analysed. [5, 7, 10, 11, 15, 19, 21]. It is necessary for RCSs to be biologically compatible and well-tolerated by the periradicular tissues [7, 10, 11, 15]. Hence, new materials and the ones already in the market should be constantly tested. This study investigated the composition, selective physicochemical properties, also the cytotoxicity of four RCSs. The null hypothesis which defined that different RCSs would show similar performance was rejected since different RCSs showed different results according to methods used.

The identification of major constituents is important for understanding biological, physical, chemical and mechanical properties of a material [7, 24-26]. EDX analysis revealed high peaks of Si, Ca, Bi, C and O for MTA Fillapex; Ca, Bi, O and C for Sealapex; Zr, W, C and O for AH-Plus; and Zn, Bi, O and C for Endofill. Silicon dioxide (silica) converts to silicon through reduction with carbon. The solubility of silica depends on its crystalline form that often shows exceptional mechanical properties (strength, hardness, fracture toughness). Calcium oxide is one of the most important compounds in MTA Fillapex and Sealapex. and thus, calcium ions were most commonly found, which was in accordance with the literature [17]. Bismuth is added to a material to improve its radiopacity and alter the setting time, hydration reaction, porosity and density of the material [8, 9]. Zirconium is mainly used as opacifier and improves the corrosion resistance of the material [8]. Tungsten (in calcium-tungstate form) is associated with the radiopacity of the material [8].

Recent published studies found slightly different chemical compositions [25, 27]. Borges *et al.* investigated the changes in the structure of the surface of RCSs after a solubility test [27]. EDX analysis of MTA Fillapex revealed that its external surface was initially composed of C, Zr, O, W, Ca, and Si. The external surface of Sealapex was originally composed of O, Bi, Ca, C, Zn, titanium (Ti), Si and Zr. In AH-Plus, C, Zr, W, Bi and Ca were identified before the solubility test. Sampaio *et al.* [25] observed that Fillapex was composed of Si, Ca, and Bi, whilst Sealapex was formed by Ca and Bi, AH-Plus by Zr, W and Ca, and EndoFill by Zn, Bi and barium (Ba). The difference between thementioned studies might be explained by variations in experimental conditions. In addition, some of the tested root canal sealers were paste-to-paste sealers, and some components might get deposited at the lower end of the tube [28].

The irritation of periapical tissues and gaps between root canal walls and obturation materials are strictly related to the high solubility of RCSs [29]. Regarding solubility, data obtained from AH-Plus and MTA Fillapex groups were in agreement to ANSI/ADA recommendations [22]. According to the given standards, a RCS should not exceed 3% of the initial mass when the solubility of the set material is tested.

The untreated group (cell control without eluate resin) was considered equal to 100%. Different superscript letters in same column represents statistically significant difference (*P*<0.05).

Nevertheless, Sealpex showed the highest solubility, followed by Endofill. Therefore, both sealers did not fulfill the solubility requirements of ANSI/ADA specification #57 [22]. The high solubility of Sealapex is related to the presence of calcium hydroxide, which produces a porous matrix with high water absorption and calcium release [17]. In Endofill, the solubility is associated with the continuous loss of eugenol from the sealer matrix by lixiviation [30] and the high solubility of sodium tetraborate in the composition. The addition of a natural resin reduced the solubility of this cement [30, 31].

Table 4. Percentage of cell viability assessed after 24 h

Material	% Mean (SD)
AH-Plus	66.23 (0.11) ^{a}
Endofill	11.25 (0.43) ^{b}
MTA Fillapex	- (0.11) ^{c}
Sealapex	- (0.02) ^{c}

AH-Plus showed the lower solubility and it can be explained by presence of a cross-linked polymer [32], related to higher stability compared to acid-base and water based cements. MTA Fillapex contains silica matrix (salicylate, diluting and natural resin) that is the insoluble component of MTA and maintains its integrity in an aqueous environment [3].

All the tested RCSs promoted an alkaline pH with values ranging from 7.01 to 10.34 which stayed high until the end of the experiment. The pH of MTA Fillapex was significantly higher during all the time periods of the test; a condition that is well-explained by the calcium oxide. Calcium oxide, upon contact with water, is converted to calcium hydroxide, dissociating into calcium and hydroxyl ions [33]. Immediately after the RCS comes in contact with water, the reaction takes place and a saturated calcium hydroxide medium is observed [17, 20, 34]. Such an alkaline environment is responsible for alkaline phosphatase activation and neutralization of acids secreted by osteoclasts; both related to the healing process [10]. The high pH also has a destructive effect on bacterial cell membranes and protein structure, which can be related to potential cytotoxicity [21].

The electrical conductivity is related to the solubility of the material. The substances, which are more soluble in water, are the first to release ions into the medium [29]. Moreover, the solubility of individual components increases over time as the "time of contact" with the solvent increases [29]. Considering the time, MTA FillApex presented significantly higher values over the time periods of the tests. In our study, the solution was not changed once the samples were immersed.

Digital images of the tested sealers were acquired using a phosphorus plate system and a scanning, capturing and reading digital device [19]. In this study, although the radiopacity of all root canal sealers was found to be in agreement to ANSI/ADA specifications [22], AH-Plus presented significantly higher values. The amount and proportion of radiopacifying agents is a determinant to define the radiopacity of a material [8]. Different radiopacifying agents are associated with different materials. Some radiopacifying agents, from the least to the most radiopaque, are described as follows: zinc oxide, barium sulfate, calcium tungstate, zirconium oxide and bismuth oxide [8]. AH-Plus cement has calcium tungstate, iron oxide and zirconium oxide in its composition as radiopacifying agents, thus, it should increase radiopacity values [5]. MTA Fillapex has bismuth oxide. In Sealapex, the presence of zinc oxide and bismuth trioxide in its formulation indicates radiopacity [9]. Endofill contains barium sulphate, zinc oxide and bismuth subcarbonate [35]. It is determinate to understand interactions of materials with biological systems (biocompatibility and cytotoxicity analysis) before clinical use [7, 10, 11, 25]. A cytotoxic sealer can interfere in periapical tissue repair [10, 11]. Factors, such as chemical constitution, may also affect cell adhesion and biocompatibility [25]. Thereby, regarding cytotoxicity, and in comparison, AH-Plus presented higher values of cell viability while all the other sealers were cytotoxic. However, an increase in apoptosis, also in oxidative stress and genotoxicity markers in human dental pulp stem cells associated with use of AH-Plus, has been reported [26].

The results of our present study have led to a more comprehensive understanding of the interactions which occur between some RCSs and tooth and periapical tissues. This extended comprehension should help investigators to design new products, with well-defined properties.

Conclusion

Endofill and Sealpex presented solubility values over those the ANSI/ADA standardization. All the tested RCSs promoted an alkaline pH during the experiment; however, MTA Fillapex presented the highest mean value for pH. Also, from the first day of the experiment to the last, MTA Fillapex presented the highest value for electrical conductivity. The radiopacity values of all RCSs were in line with ANSI/ADA standards. AH-Plus showed to be less cytotoxic than other tested RCSs.

Conflict of Interest: 'None declared'.

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