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Endodontic sealers after exposure to chlorhexidine digluconate: An assessment of physicochemical properties

Vasileios Kapralos [a,*], Josette Camilleri [b], Andreas Koutroulis [a], Håkon Valen [c], Dag Ørstavik [a], Pia Titterud Sunde [a]

[a] *Department of Endodontics, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Box 1109 Blindern, 0317 Oslo, Norway*
[b] *School of Dentistry, Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom*
[c] *Nordic Institute of Dental Materials (NIOM), Sognsveien 70 A, 0855 Oslo, Norway*

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ABSTRACT

Objectives: Final root canal irrigation should ideally maintain the physicochemical stability of root canal sealers. We seek to assess the effect of contact with 2% chlorhexidine digluconate (CHX) on the physicochemical properties of AH Plus, BioRoot™ RCS, and Pulp Canal Sealer (PCS).

Methods: Mixed sealers were placed in cylindrical teflon molds and allowed to set for 1.5x the manufacturers' setting time. Half of the specimens had their free surface in contact with CHX for the first minute of their setting period. Solubility, radiopacity, surface roughness, microhardness and wettability of the sealers were assessed up to 28 days after setting. Elemental analysis of sealer surfaces and their leachates together with pH measurements were also performed. Appropriate parametric and non-parametric analysis with post hoc tests were performed ($p < 0.05$).

Results: Exposure to CHX had no effect on solubility and radiopacity of all sealers. CHX altered the surface roughness of PCS and BioRoot RCS ($p < 0.05$). Contact with CHX reduced the microhardness of AH Plus and PCS ($p < 0.05$). AH Plus was more hydrophilic after CHX contact, whereas PCS became more hydrophobic ($p < 0.05$). AH Plus and PCS surfaces appeared to adsorb CHX as exhibited by chlorine peaks after contact with CHX. Sealer leachates' alkalinity was not affected. CHX increased elution of silicon and zirconium for BioRoot and zinc for PCS leachates.

Significance: In our study, CHX affected sealers' physicochemical properties to various extents. Further studies are needed to confirm the obtained results by investigating various final irrigation strategies and correlating to biological properties.

1. Introduction

The sealing of root canals in endodontic treatment is a combination of a core material and sealer, where the sealer fills the gap between the core and the root canal walls [1]. Single cone obturation techniques are more dependent on sealer properties since the root filling has a large volume of sealer. Several root canal sealers with various chemistries have been developed and used. Physical and chemical properties of endodontic sealers should remain consistent in the long term to secure the three-dimensional hermetic filling/sealing of the root canals [2].

Various irrigation solutions are used prior to root canal filling [3,4]. After completion of chemo-mechanical root canal preparation, remnants of irrigation solutions are present in the root canal system [5,6]. Dental practitioners may sometimes face challenges in adequately drying the canals, especially in the apical third of the root canal or in cases of anatomical irregularities. This can result in potential movement of fluids toward the apical foramen after drying, or the inability to sufficiently dry the apical portion of the root canal using paper points. Chlorhexidine digluconate (CHX) 2% is commonly used in endodontic treatment as final irrigant, making it a candidate irrigation fluid with the potential to interact with the dentin and sealers in the root canal system [7]. CHX is a cationic substance that possesses broad antimicrobial properties and has both bacteriostatic and bactericidal effects depending on its concentration [8,9]. CHX has the ability to bind to hard dental tissues (substantivity) and confers lasting antimicrobial properties to dentine [8,9]. The presence of CHX on the dentine surface and its gradual release

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[*] Correspondence to: Geitmyrsveien 71, 0455 Oslo, Norway.
E-mail addresses: vasilis.kapralos@gmail.com, vasileios.kapralos@odont.uio.no, vasileios.kapralos@zzm.uzh.ch (V. Kapralos).

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may also modify the sealers' properties.

Sealer solubility may lead to a lack of integrity in the material which in turn may compromise the technical quality of an endodontic treatment [10]. This loss of structure creates gaps in the material bulk and along the sealer/dentin or sealer/gutta-percha interface [11] which may create a pathway for microbes and their products into periapical tissues and jeopardise the healing process [10]. In addition, a soluble sealer may be subject to degradation that may further risk its chemical stability and affect other of its properties [12]. Furthermore, leaching of chemicals such as eugenol from zinc-oxide eugenol sealers may be irritating to the periapical tissues and increase cytotoxic effects [13].

To date, the effect of the irrigation used prior to root filling has not been investigated in depth [14,15]. Most studies have investigated the effect of irrigation solutions on sealers' properties such as solubility, sealing ability, microleakage, and wettability [16–19] especially on epoxy resin-based sealers. Newer studies have included calcium silicate-based sealers in their comparisons and focused on other properties such as antimicrobial activity and physicomechanical behaviour [15,20]. The presence of irrigants inside the root canal may affect the sealer chemistry particularly with reactive materials like calcium silicate-based sealers. Given that most modern single cone obturation techniques heavily rely on sealer properties, and newer calcium silicate-based sealers are widely used in endodontic treatments, exploring the interactions between irrigation fluids and these newer endodontic sealers may be clinically significant. CHX is mainly used as final irrigation [7] which may in turn affect both dentine and the sealer placed after chemomechanical preparation. The immediate placement of sealers after the last irrigation with CHX and its ability to be gradually released over time (substantivity) [8,9] makes CHX possibly interact with the sealers used in root canal obturation.

The aim of the study was to assess the physical properties (solubility, radiopacity, wettability, microhardness, surface roughness) and chemical properties (pH assessment, elemental analysis/chemical characterisation of both sealer surfaces and leachates) of sealers with and without CHX contact as well as to visually evaluate their surfaces macro- and microscopically. The null hypothesis tested was that the sealers' properties would not be affected by exposure to CHX.

2. Materials and methods

An epoxy resin-based sealer, AH Plus (Dentsply International Inc, York, PA, USA), a tricalcium-silicate based sealer, BioRoot™ RCS (Septodont, Saint-Maur-des-Fossés, France), and a zinc oxide eugenol sealer, Pulp Canal Sealer (PCS) (Kerr Corporation, Romulus, MI) were tested. The materials were mixed according to manufacturers' instructions.

Chlorhexidine digluconate, 20% in water solution, (Lot # BCBS7878V, Sigma-Aldrich, St. Louis, MO, USA) was diluted in sterile distilled water (water) and standardized to 2%.

2.1. Sample preparation

The physicochemical properties of sealers with and without CHX contact were assessed with the use of cylindrical specimens (diameter: 10 mm; height: 1 mm for radiopacity, 1.5 mm for solubility) (Fig. S1a). The sealers were allowed to set into cylindrical teflon moulds with bottom and side walls in such way to cover the bottom face and side surfaces of the sealer samples and leave free the top face of the materials (Fig. S1a). This mould design enabled to isolate the bottom and side faces of the sealers with teflon and expose only the upper sealer surface, which was processed for testing. The sealers placed in the moulds were either allowed to set independently (no CHX) or in contact with CHX. For CHX exposure group, a drop of 25 µl CHX was applied upon half of the sealer samples with a pipette and evenly spread with a sterile plastic inoculation loop (Fig. S1b). After 1 min of contact with CHX, the drop was aspirated with a pipette (Fig. S1b) and the sealers were placed in a dry incubator at 37 °C for 20 min to let any excess liquid dry out before being allowed to set (Fig. S1c). The power calculation using G*Power 3.1 (Heinrich Heine University, Düsseldorf, Germany) [21] for determining the sample size for each assay and experimental condition indicated at least seven samples in each assay (effect size $f = 0.40$, α error probability $= 0.05$). Thus, nine samples ($n = 9$) were used for each experimental condition.

2.2. Physical properties

2.2.1. Determination of solubility

The solubility of the sealers was tested as a percentage of the mass of specimen material removed from the distilled water compared with the original mass of the specimens. Moulds measuring 10 mm in diameter and 1.5 mm in height were used (Fig. S2a). After sample preparation, the sealers were allowed to set into the moulds for a time period 50% longer than the setting time stated by the manufacturers (t_0) and each specimen was weighed to an accuracy of ± 0.1 µg (m_1) (Fig. S2a). The specimens were placed at time point t_0 into snap vials containing 2.717 mL (the immersion ratio $\approx 28.9\ \text{mm}^2/\ \text{mL}$ per specimen applied by ISO 6876 was used as reference). The snap vials had been weighted to an accuracy of ± 0.1 µg prior to immersion to calculate their initial weight (m_i) (Fig. S2a). After 24 h of incubation, the sealer surfaces of the specimens were rinsed with 2 mL water, and the washings were allowed to drain back into the snap vials (Fig. S2d). Subsequently, the snap vials were placed in an oven at 110 °C for 24 h and afterwards weighed again (m_f) (Fig. S2d). The procedure was repeated for the same specimens; however, the specimens were now stored in distilled water for up to 4 weeks (Fig. S2e). The solubility was calculated using the Eq. (1) for 5 immersion periods (t_0-1 day; 1-7 days; 7-14 days; 14-21 days; 21-28 days):

$$solubility \quad (\%) = \frac{m_f - m_i}{m_1} x100 \tag{1}$$

2.2.2. Evaluation of radiopacity

Specimens (10 mm in diameter, 1 mm in height) were also allowed to set into teflon moulds for a time period 50% longer than the setting time stated by the manufacturers (t_0) and evaluated for radiopacity after immersion into distilled water at time points (1-, 7-, 14-, 21-, 28- days) (Fig. S3b). In addition, specimens ($n = 9$ for each experimental group) with the same dimensions were prepared as aforementioned (Fig. S3b), incubated at 37 °C, 100% humidity and evaluated for radiopacity as freshly mixed and at the same time points (1-, 7-, 14-, 21-, 28- days) (Fig. S3c). Specimens were arranged on a photo-stimulable phosphor plate (VistaScan image plate 4 +, Durr Dental, Bietigheim-Bissingen, Germany) adjacent to a calibrated aluminium step wedge with 3 mm increments. A standard X-ray machine (Soredex MinRay, KaVo Dental, Germany) was used to irradiate the specimens using an exposure time of 0.50 s at 10 mA, tube voltage at 65 ± 5 kV and a cathode–target film distance of 300 ± 10 mm. The radiographs were then processed (VistaScan Mini View, Durr Dental, Bietigheim-Bissingen, Germany) and a digital image of the radiographs was obtained. For interpretation of results, a method previously described was used [22]. Briefly, an imaging programme, ImageJ (Rasband WS, ImageJ; US National Institute of Health, Bethesda, MD, USA) was utilised to calculate the grey pixel value on the radiograph of each step in the step-wedge. Consequently, data for the thickness of the aluminium against the grey pixel value on the radiograph was plotted; the best-fit logarithmic trend line was then identified.

2.2.3. Wettability and microhardness assessment

The immersed sealer specimens ($n = 9$, 10 mm in diameter and 1.5 mm height) from solubility assay were further tested for wettability (Fig. S4a) and microhardness (Fig. S4b). Additionally, sealer specimens in teflon moulds of the same dimensions (10 mm in diameter, 1.5 mm in height) were allowed to set at 37 °C, 100% humidity with and without CHX contact and after 28 days were assessed for the abovementioned properties (Figs. S4a and 4b).

A 20 µl drop of distilled water was placed with a syringe on the surface of the sealer samples, and the contact angle was measured using a contact angle goniometer (model 100–00, ramé-hart, USA).

Microhardness testing was performed by applying an indentation technique (Vickers test), with the use of a hardness-testing instrument (Duramin 40, Struers, Rødevre, Denmark). A pyramidal square-based diamond indenter was applied onto the sealer surfaces with a load ranging up to 300 gf for a dwell time of 15 s. At least two independent indentations at a minimum distance of 5 mm selecting non-overlapping microscopical regions were performed on each sample and the Vickers hardness number (VHN) was recorded.

2.2.4. *Surface roughness assessment*

The sealer specimens used in radiopacity assays were further assessed for surface roughness (Fig. S4c). The sealer specimens were mounted upon carbon tapes and imaged in the SEM (TM4000Plus II, Hitachi, Tokyo, Japan). Four backscattered images were obtained at 4 independent sections of each sealer specimen with 4 different tilt angles at 100 × magnification. Stereoscopic reconstruction in a 3D model of these images was performed with the use of a suitable software (MountainsMap 8; Digital Surf, Besançon, France). Surface roughness values were calculated following calibration of the programme based on a reference angle (60°) artificially induced upon the surface of each sealer.

2.3. *Chemical properties*

2.3.1. *Elemental analysis of sealer surfaces*

The sealer samples viewed in SEM and analysed for surface roughness (samples derived from radiopacity assay) were further chemically characterised by means of EDS (Fig. S4c). High magnification EDS elemental analysis was carried out at 15 kV and a working distance of 10 mm. EDS was performed in both spot and rectangular areas of the sealers' surface. Additionally, elemental maps at the same levels were obtained and each element was marked out/ designated in a different color.

2.3.2. *Assessment of pH*

The sealers' alkalinity in contact or not with CHX was assessed measuring the pH of sealers' leachates derived from solubility test (Fig. S2c) after 1, 7, 14, 21 and 28 days. The pH values were assessed with a pH meter (Sension+ PH31; Hach, Loveland, CO, USA), previously calibrated using buffer solutions of pH 4, 7, and 14.

2.3.3. *Elemental analysis of sealer leachates*

Sealer specimens were formed as it was aforementioned using teflon moulds (10 mm in diameter, 1.5 mm in height) (Fig. S5a). Two groups were formed according to exposure to CHX: group 1, no CHX (no contact); group 2, CHX (short-term exposure: 1 min contact time) (Fig. S5b and c). The caps with the sealers were immersed in 3 mL distilled water solution and leachates from freshly mixed (2 h) and 24-hours set sealers were allowed to form for 24 h and 28 days respectively (Fig. S5d). Thus, two leaching periods were tested: "Freshly mixed-1 day" and "1–28 days". Following leaching process, the leachates were filtrated under sterile conditions (Fig. S5e) and processed for ICP- OES by Sheffield Analytical Services (Sheffield, UK) (Fig. S5f).

2.4. *Macro- and microscopical inspection of the sealers*

Macroscopical evaluation (colour assessment and macrostructural evaluation of the surfaces) of the sealer samples used for radiopacity assay was performed by photographing the samples with the use of a DSLR camera (Nikon D3300, Nikon, Tokyo, Japan).

Scanning electron microscopy (SEM) was also performed on the aforementioned sealer samples. Briefly, the specimens were mounted upon carbon tapes and viewed with the scanning electron microscope (TM4000Plus II, Hitachi, Tokyo, Japan). Accelerating voltage ranged between 5 and 15 kV and high magnification micrographs were captured at the backscattered or secondary electron mode to assess the surface characteristics.

2.5. *Statistical analysis*

The statistical analysis was performed with IBM SPSS Statistics software version 28 (IBM, Armonk, USA). Before each statistical analysis, the data were assessed for normality with the Shapiro-Wilk test and homogeneity of variance with Levine's test. Statistical analysis of the solubility, wettability, microhardness and pH (normally distributed) was performed using one-way ANOVA and Dunnett's C post hoc multiple comparison test (for unequal variances across groups) ($p < 0.05$). For radiopacity, one-way ANOVA (normally distributed and equal variances across groups) was performed using Bonferroni's multiple comparisons test. In case of pairwise comparisons of two groups, parametric t-tests were performed ($p < 0.05$). For solubility, radiopacity and pH assessment the comparisons were performed as follows:

- within the same sealer and experimental condition, between different immersion periods
- within the same immersion period, between different sealers and experimental conditions

The surface roughness and elemental analysis (ICP) were analysed using the nonparametric Kruskal–Wallis and Dunn's test due to absence of normal distribution of data ($p < 0.05$). In case of pairwise comparisons of two groups, nonparametric t-tests were performed ($p < 0.05$).

3. Results

3.1. *Physical properties*

3.1.1. *Determination of solubility*

No statistically significant differences were observed for all sealers tested with and without exposure to CHX within each immersion period tested ($p > 0.05$). BioRoot RCS exhibited statistically higher solubility during t_o- 1 day compared to the following immersion periods tested both with and without CHX contact ($p < 0.05$). Data for solubility are shown in Table 1.

3.1.2. *Evaluation of radiopacity*

No statistically significant differences were observed for all three sealers with and without exposure to CHX both for immersed and non-immersed samples in all ageing periods ($p > 0.05$). Non immersed BioRoot RCS with and without CHX contact exhibited statistically higher radiopacity in all ageing periods compared to the immersed samples ($p < 0.05$). Data for radiopacity are shown in Fig. 1.

3.1.3. *Wettability, microhardness and surface roughness assessment*

AH Plus and PCS were hydrophobic while BioRoot was highly hydrophilic as complete wetting (contact angle at 0°) of its surfaces was observed in all conditions tested. Non-immersed AH Plus and PCS presented higher contact angles compared to immersed samples for both CHX and no CHX contact ($p < 0.05$). Contact with CHX further decreased contact angles in both immersed and non-immersed AH Plus ($p < 0.05$). As for PCS, CHX rendered the sealer more hydrophilic only for the non-immersed samples ($p < 0.05$).

AH Plus exhibited the highest microhardness for all conditions tested. Contact with CHX reduced the microhardness of immersed AH Plus ($p < 0.05$). The microhardness of PCS was compromised by CHX both in immersed and non-immersed samples ($p < 0.05$) compared to no

Table 1
Mean solubility values with standard deviation for test sealers with and without CHX contact. Read horizontally (within the same sealer and experimental condition, between different immersion periods, Tukey's multiple comparison test) and vertically (within the same immersion period, between different sealers and experimental conditions, parametric t-tests and Dunnett's C multiple comparison test), the same superscript letter shows no statistically significant differences, $p > 0.05$.

Material	Condition	Solubility				
		t_0-1 day	1-7 days	7-14 days	14-21 days	21-28 days
AH Plus	No CHX	0.0 (0.0)^a	0.0 (0.0)^a	0.0 (0.0)^a	0.0 (0.0)^a	0.1 (0.0)^a
	CHX	0.1 (0.1)^a	0.0 (0.1)^a	0.0 (0.0)^a	0.0 (0.0)^a	0.1 (0.0)^a
BioRoot RCS	No CHX	15.8 (9.9)^b	3.5 (1.9)^c	2.6 (1.4)^{cd}	2.1 (1.2)^d	1.6 (1.1)^d
	CHX	17.1 (5.9)^b	3.4 (0.4)^c	1.91 (0.7)^d	1.0 (0.6)^d	0.5 (0.6)^d
PCS	No CHX	0.1 (0.0)^a	0.1 (0.0)^a	0.1 (0.0)^a	0.1 (0.0)^a	0.1 (0.1)^a
	CHX	0.1 (0.0)^a	0.1 (0.0)^a	0.1 (0.0)^a	0.1 (0.0)^a	0.0 (0.0)^a

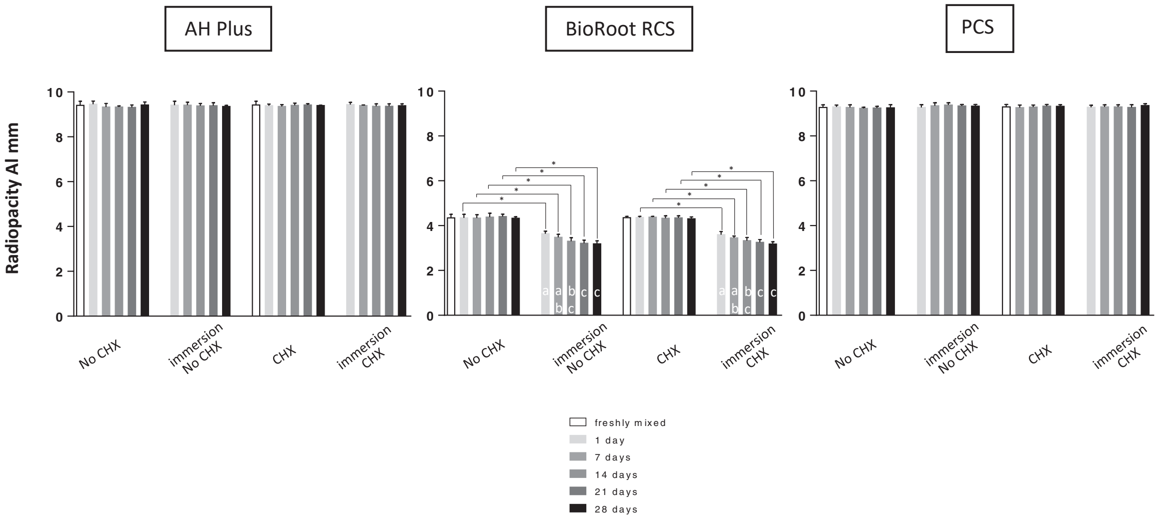

Fig. 1. Mean radiopacity values of sealers with standard deviation expressed in mm Al. The same letter indicates no statistically significant differences in radiopacity between the different ageing times within the same sealer and condition tested. Asterisks upon the brackets signify statistically significant differences between no CHX and CHX contact within the same sealer, same ageing times and condition tasted. One-way ANOVA (normally distributed and equal variances across the groups) was performed using Bonferroni's multiple comparisons test.

contact group, while BioRoot RCS remained unaffected.

Surface roughness was increased in AH Plus after immersion compared to non-immersed samples both with and without CHX contact ($p < 0.05$). Regarding BioRoot RCS, the immersed samples exhibited lower surface roughness than the non-immersed ones ($p < 0.05$). Contact with CHX reduced the surface roughness of non-immersed BioRoot RCS ($p < 0.05$), whereas no effect was observed for the immersed samples. PCS without CHX contact after immersion showed higher surface roughness compared to non-immersed samples ($p < 0.05$). On the contrary, the immersed PCS with CHX contact presented lower surface roughness compared to non-immersed samples. Contact with CHX increased the surface roughness of non-immersed PCS whereas it reduced it for immersed samples ($p < 0.05$). Data for wettability, microhardness and surface roughness are shown in Table 2. Representative images acquired after stereoscopic reconstruction of scanning electron micrographs obtained with 4 different tilt angles are shown in Fig. 2.

3.2. *Chemical properties*

3.2.1. *Elemental analysis of sealer surfaces*

AH Plus with CHX contact exhibited extra peaks for chlorine in addition to silicon, calcium, zirconium and tungsten both for immersed and non-immersed samples (Fig. 3A). BioRoot RCS had silicon, calcium, chlorine and zirconium in all conditions tested. PCS demonstrated chlorine peaks, when CHX was applied, together with zinc, and iodine peaks, which were evident in no CHX groups (Fig. 3C). The spectra of

Table 2
Contact angle, microhardness (mean and standard deviation) and surface roughness (median and 25 –75 interpercentile range) for 28 days, non-immersed and immersed sealers with and without CHX contact. Read vertically (between different sealers and experimental conditions), the same superscript letter shows no statistically significant differences, $p > 0.05$.

Group	Contact angle (°)	Microhardness (VHN)	Surface roughness (Ra)
AH Plus_*No immersion*			
No CHX	83.8° (1.4)^a	25.28 (2.81)^a	0.052 (0.029)^a
CHX	66.2° (6.7)^b	23.42 (1.29)^a	0.061 (0.028)^a
AH Plus_*immersion*			
No CHX	71.4° (1.6)^c	30.62 (3.80)^b	0.154 (0.081)^{ab}
CHX	59.0° (1.7)^d	20.88 (1.27)^c	0.221 (0.112)^b
BioRoot RCS_*No immersion*			
No CHX	0.0° (0.0)^e	9.50 (0.77)^d	4.482 (1.078)^c
CHX	0.0° (0.0)^e	10.31 (1.09)^d	2.579 (1.203)^d
BioRoot RCS_*immersion*			
No CHX	0.0° (0.0)^e	8.02 (2.04)^d	1.742 (0.958)^e
CHX	0.0° (0.0)^e	9.91 (1.97)^d	1.539 (0.974)^e
PCS_*No immersion*			
No CHX	73.2° (2.0)^c	14.61 (0.52)^e	0.309 (0.210)^b
CHX	87.5° (4.7)^f	11.18 (1.07)^d	3.329 (0.739)^{fcd}
PCS_*immersion*			
No CHX	55.8° (6.2)^d	16.22 (1.43)^e	2.280 (2.259)^{ge}
CHX	59.5° (2.6)^d	6.51 (1.82)^f	1.155 (0.537)^{ge}

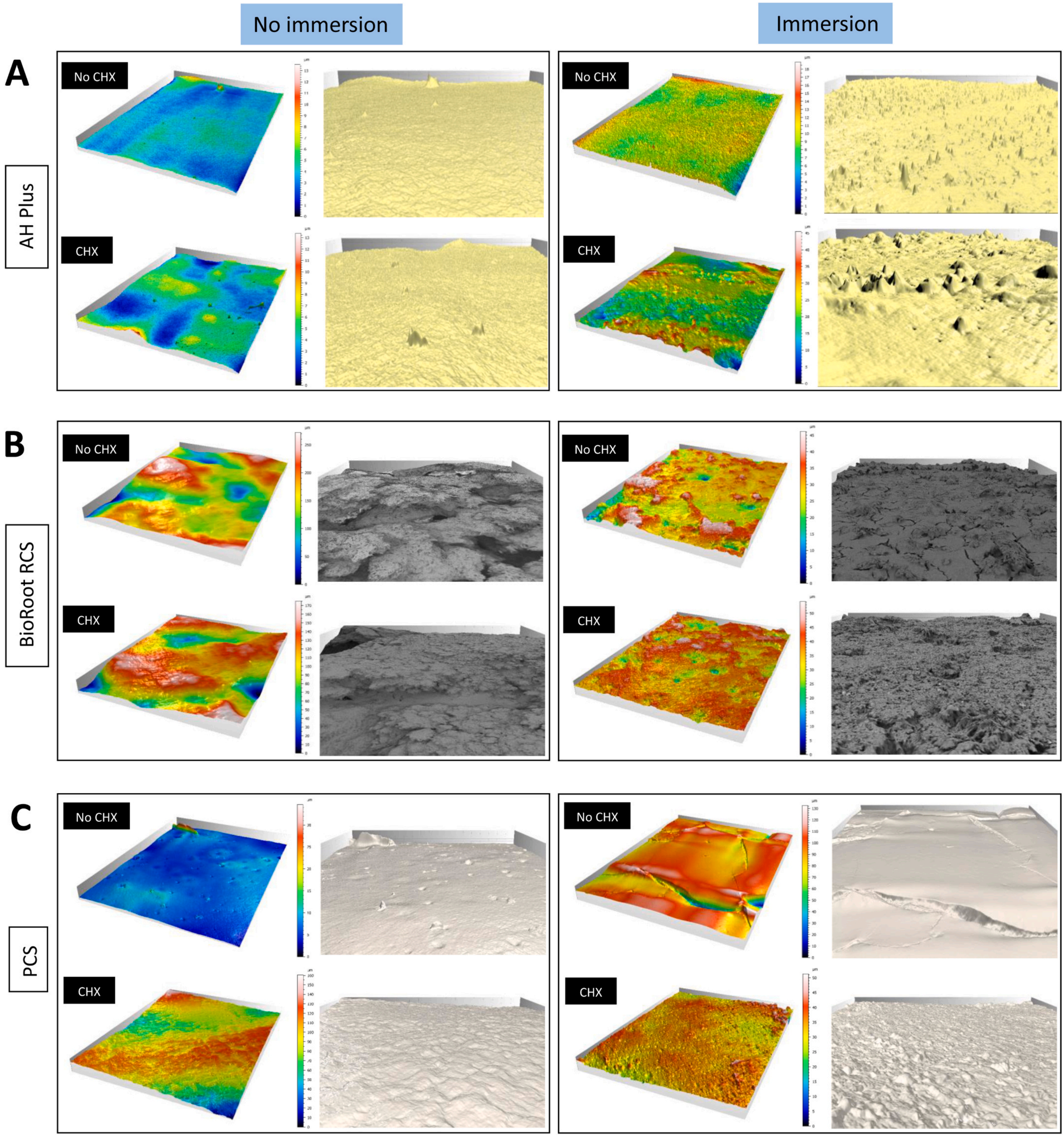

Fig. 2. Representative images acquired after stereoscopic reconstruction of scanning electron micrographs obtained with 4 different tilt angles. Sealers were also exposed to CHX as well subjected to immersion in water: AH Plus (A); BioRoot RCS (B); and PCS (C).

elemental analysis are shown in Fig. 3 and representative elemental maps in Supplementary Material 2.

3.2.2. Sealer leachates - Assessment of pH

BioRoot RCS had the highest pH for all the setting times (1, 7, 14, 21 and 28 days) of the sealers with and without CHX contact ($p < 0.05$). AH Plus at 1 day with and without CHX contact presented the highest pH with a decreasing trend over setting time, whilst CHX did not affect the pH values for each setting time tested compared to AH Plus alone. No significant differences were found between PCS alone and with CHX contact for all setting times tested ($p > 0.05$). Data for alkalinity are shown in Table 3.

3.2.3. Elemental analysis of sealer leachates

For AH Plus in contact with CHX, "1–28 days" samples leached in solution more calcium than the "freshly mixed-1 day" samples ($p < 0.05$). No statistically significant differences were reported for all other conditions tested (with/without CHX contact and leaching period)

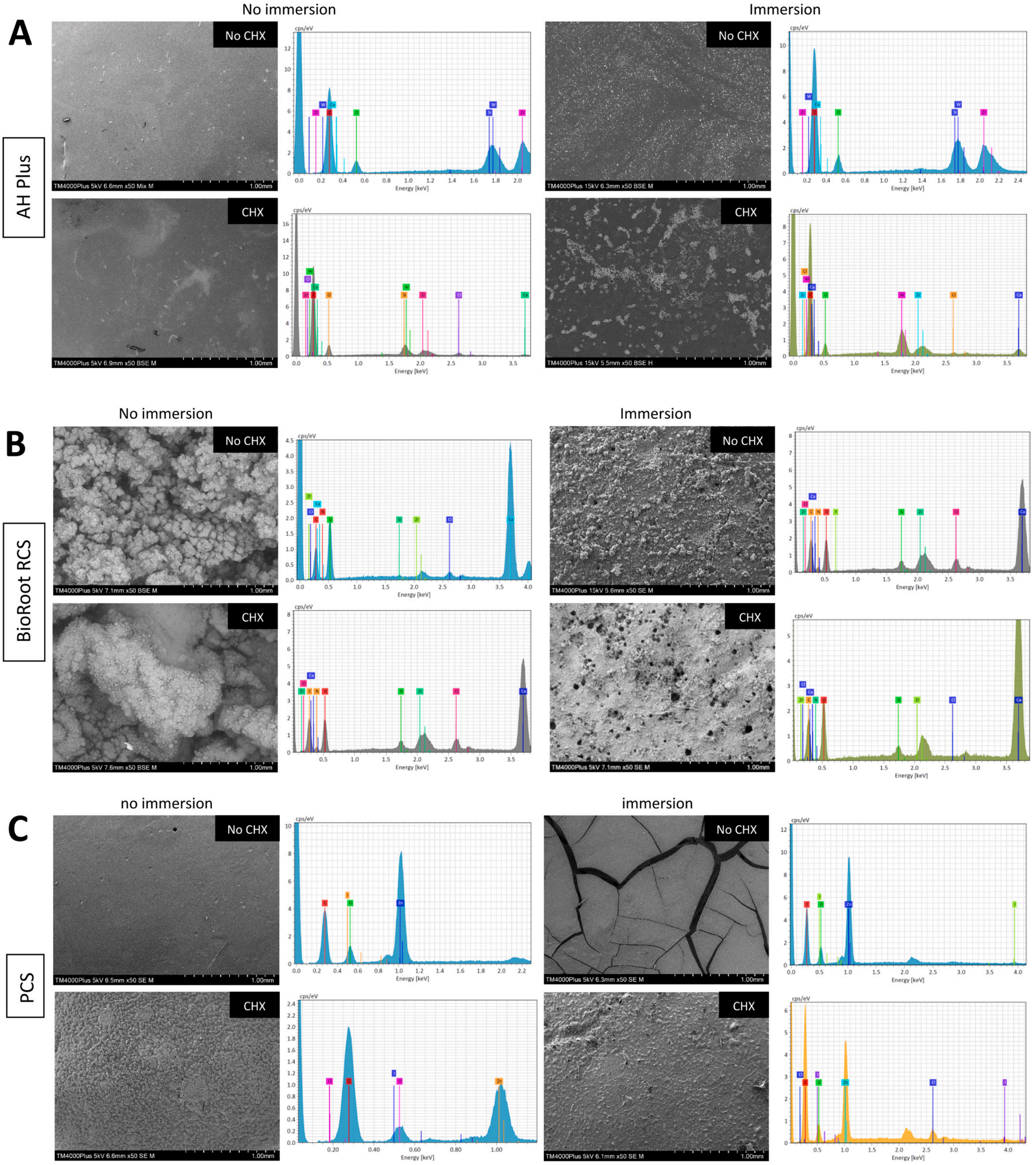

Fig. 3. High magnification scanning electron micrographs (50 ×) of tested sealers after no or CHX contact with and without immersion to water. Elemental analysis of sealer surfaces and their spectra.

for all elements analysed. BioRoot RCS with and without CHX presented no differences in calcium ion release. The silicon and zirconium ion release were higher in contact with CHX compared to no contact in freshly mixed-1 day BioRoot ($p < 0.05$). Moreover, the freshly mixed-1 day BioRoot RCS leached significantly more silicon and zirconium ions compared to 1–28 days samples ($p < 0.05$). PCS in contact with CHX eluted significantly higher amount of zinc compared to no contact for both leaching periods tested. Zinc was eluted in low quantities by “freshly mixed-1 day” PCS compared to “1–28 days” ($p < 0.05$). The release of silver and aluminium was independent of CHX contact and leaching period ($p > 0.05$). The data for elemental analysis are shown in Table 4.

Table 3
Mean pH values and standard deviation of sealers' leachates in contact or not with CHX (pH= 5.98 ± 0.11). Distilled water (6.89 ± 0.15) used as the extraction vehicle. Read horizontally (within the same sealer and experimental condition, between different immersion periods, Tukey's multiple comparison test) and vertically (within the same immersion period, between different sealers and experimental conditions, parametric t-tests and Dunnett's C multiple comparison test), the same superscript letter shows no statistically significant differences, $p > 0.05$.

Material	Condition	pH (distilled water: 6.89 ± 0.15)				
		1 day	7 days	14 days	21 days	28 days
AH Plus	No CHX	7.47 (0.25)^{a}	7.28 (0.29)^{ab}	7.20 (0.13)^{b}	7.10 (0.16)^{b}	7.21 (0.23)^{b}
	CHX	7.38 (0.13)^{ab}	7.23 (0.17)^{ab}	7.28 (0.08)^{ab}	7.22 (0.13)^{ab}	7.31 (0.16)^{ab}
BioRoot RCS	No CHX	12.45 (0.16)^{c}	12.49 (0.04)^{c}	12.47 (0.07)^{c}	12.45 (0.17)^{c}	12.29 (0.19)^{c}
	CHX	12.20 (0.04)^{cd}	12.28 (0.09)^{cd}	12.30 (0.15)^{cd}	12.05 (0.18)^{de}	11.83 (0.21)^{e}
PCS	No CHX	6.98 (0.12)^{f}	6.85 (0.11)^{f}	7.03 (0.14)^{fb}	7.00 (0.19)^{fb}	7.18 (0.11)^{fb}
	CHX	6.87 (0.10)^{f}	7.01 (0.07)^{f}	7.18 (0.07)^{fb}	7.13 (0.06)^{fb}	7.14 (0.05)^{fb}

Table 4
Mean and standard deviation of elements based on the ICP analysis for sealers with and without CHX contact for "freshly mixed – 1 day" and "1–28 days" immersion periods. Read horizontally, the same small superscript letter indicates no statistically significant differences between "no CHX" and "CHX" contact groups within the same material and element tested ($P < 0.05$). Read vertically, the same capital letter shows non-statistically significant difference between the two different immersion periods "freshly mixed – 1 day" and "1–28 days" for the exact same conditions of testing (CHX contact and element) ($P < 0.05$).

AH Plus	Ca		Si		W		Zr	
	No CHX	CHX	No CHX	CHX	No CHX	CHX	No CHX	CHX
Freshly mixed - 1 day	0,51 (0,08)$^{a-A}$	0,42 (0,09)$^{a-A}$	0,27 (0,05)$^{a-A}$	0,23 (0,03)$^{a-A}$	1,92 (0,42)$^{a-A}$	1,14 (0,35)$^{a-A}$	<0,01^{a-A}	<0,01^{a-A}
1–28 days	0,72 (0,15)$^{a-A}$	0,93 (0,09)$^{a-B}$	0,46 (0,02)$^{a-A}$	0,45 (0,07)$^{a-A}$	1,62 (0,45)$^{a-A}$	1,9 (0,34)$^{a-A}$	<0,01^{a-A}	<0,01^{a-A}

BioRoot RCS	Ca		Si		Zr	
	No CHX	CHX	No CHX	CHX	No CHX	CHX
Freshly mixed - 1 day	2862 (633,4)$^{a-A}$	2386 (116,4)$^{a-A}$	0,79 (0,08)$^{a-A}$	8,65 (9,8)$^{b-A}$	0,03 (0,03)$^{a-A}$	3,49 (5,34)$^{b-A}$
1–28 days	2510 (360,6)$^{a-A}$	2482 (91,7)$^{a-A}$	0,39 (0,1)$^{a-A}$	0,28 (0,04)$^{a-B}$	<0,01^{a-A}	<0,01^{a-B}

Pulp Canal Sealer	Zn		Ag		Al	
	No CHX	CHX	No CHX	CHX	No CHX	CHX
Freshly mixed - 1 day	1,85 (1,15)$^{a-A}$	6,46 (2,16)$^{b-A}$	<0,1^{a-A}	<0,1^{a-A}	<0,1^{a-A}	<0,1^{a-A}
1–28 days	11,48 (1,43)$^{a-B}$	26,1 (10,3)$^{b-B}$	<0,1^{a-A}	<0,1^{a-A}	0,06 (0,1)$^{a-A}$	<0,1^{a-A}

3.3. *Macro-and microscopical inspection of the sealers*

The macroscopic characteristics of the sealers are shown in Fig. 4. No evident changes in non-immersed AH Plus surfaces were shown both with and without CHX contact along the ageing time. The immersed AH Plus samples presented whitish depositions which were more apparent in contact with CHX (Fig. 4A). Crystalline spherical particles were observed over the non-immersed BioRoot RCS surfaces both with and without CHX contact whereas immersed samples presented flat surfaces with few evident indentations (Fig. 4B). Non-immersed PCS presented flat, even surfaces with a grey background whereas contact with CHX changed the topography and the colour of the surfaces to a more yellowish hue. The immersed PCS without CHX presented dry surface texture with a significant amount of cracks in the bulk of the material, a declare of extensive shrinkage (Fig. 4C). Contact with CHX reduced the number of cracks on the surfaces, while more capillary voids were (became) evident.

Under SEM, non-immersed AH Plus with and without CHX contact did not present any characteristic features upon their surfaces; only few voids were present for AH Plus with CHX (Fig. 3A). The surfaces of immersed AH Plus were rough presenting whitish depositions, which were more apparent and organised in contact with CHX. Non-immersed BioRoot RCS surfaces with and without CHX contact were partially covered by crystal-like depositions. Immersed BioRoot RCS demonstrated many voids/pores of various sizes especially when in contact with CHX (Fig. 3B). Non-immersed PCS demonstrated an even surface without any characteristic formations, but few pores whereas CHX contact created a rougher presentation of the sealer's surface. The immersed PCS without CHX presented dry surface texture with a significant amount of cracks, similarly to macroscopic images. Contact with CHX reduced the number of cracks on the surfaces, while more voids became evident (Fig. 3C).

4. Discussion

The aim of the study was to assess the effect of exposure to CHX on the physical and chemical properties of AH Plus, BioRoot RCS and PCS. The null hypothesis was rejected as CHX affected the sealers' properties to a varying degree.

Three endodontic sealers with different chemical compositions were tested. AH Plus is an epoxy resin based endodontic sealer which has been in clinical use for many decades. The properties of the sealer are well documented, and it is often used in studies as a benchmark for comparisons [1,23]. BioRoot RCS is a hydraulic calcium silicate based sealer with both high antibacterial efficacy [20] and low cytotoxicity [24], but the environmental conditions may affect its hydraulic properties [25]. PCS is a zinc-oxide eugenol sealer which has been used for a long time in clinical practice. It has antibacterial properties [15] but also cytotoxicity attributed to eugenol release [24,26,27].

In our study design, we favored the direct application of CHX on sealers to ensure adequate contact, considering the different hydrophilicity of the sealers. When the same amount of liquid is applied to their surfaces, the sealers exhibit different degrees of wetting. For example, the same amount of CHX as a drop spreads and covers a larger portion of the BioRoot RCS surface (a hydrophilic material) compared to AH Plus and PCS (hydrophobic materials), which require larger volumes of CHX to achieve the same drop spreading and sample coverage. To address the issue of varying hydrophilicity among the tested sealers, we followed a

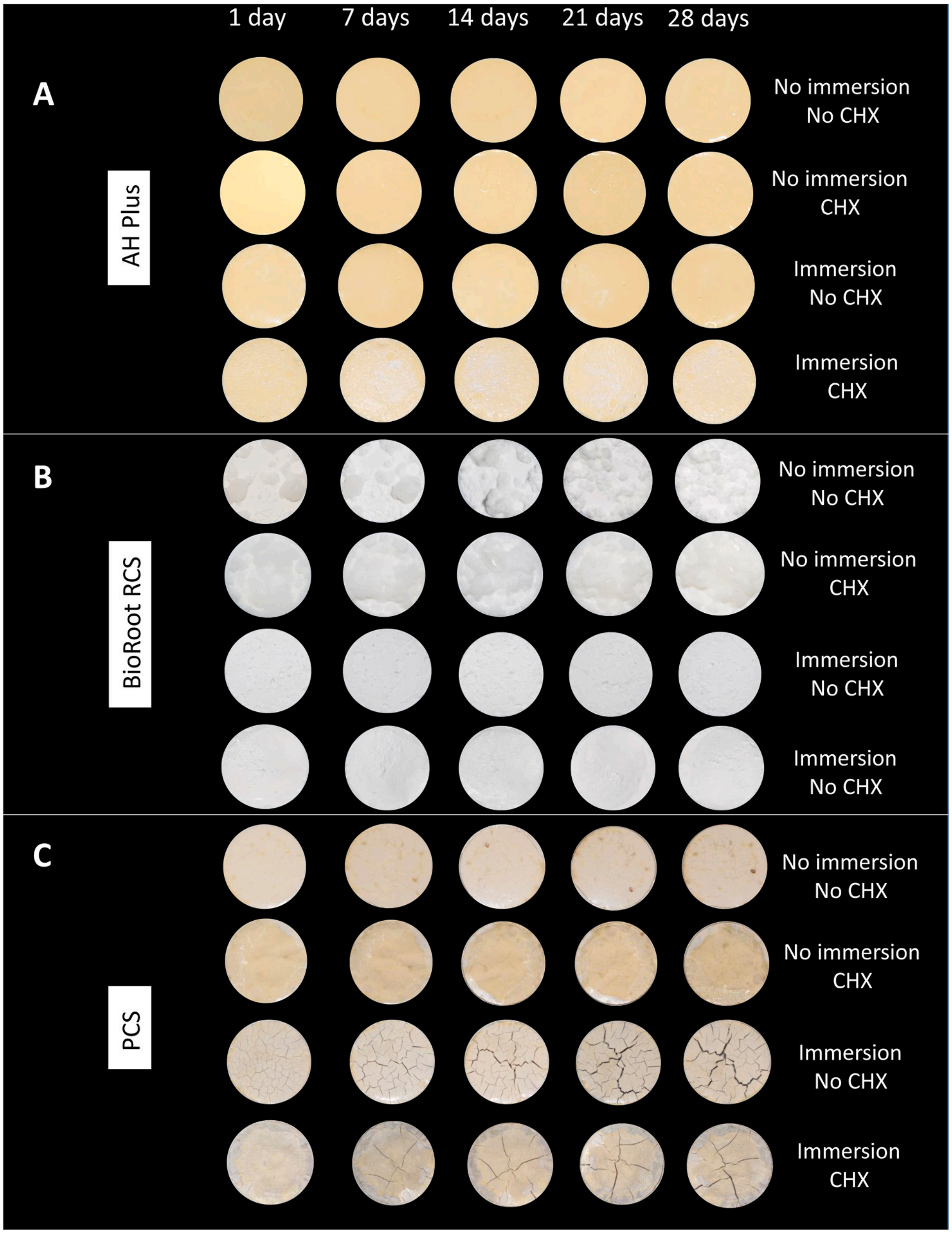

Fig. 4. Indicative images of sealers AH Plus (A); BioRoot RCS (B); and PCS (C) with and without CHX contact, with and without immersion to water.

guiding principle regarding the CHX volume and the surface area of the samples. Our goal was to use as little CHX as possible to adequately cover the sealers' surface area and mimic the clinical scenario. As mentioned and detailed in the materials and methods section, the sealers came into contact with 2% CHX as freshly mixed materials (directly after mixing following the manufacturers' guidelines) and were subsequently evaluated for their physical properties, both when freshly mixed and at different stages of setting, up to 28 days. While our study has inherent limitations as an in vitro investigation, the aspect of material contact with CHX mirrors the clinical setting, where it occurs directly when the material is fresh and is placed in the root canal system.

The radiopacity of endodontic sealers is important for assessing the technical quality of endodontic treatments. In addition, adequate radiopacity is needed to distinguish the filling from surrounding anatomical structures. Contact with irrigation solutions or tissue fluids may result in higher solubility of sealer components including radiopaque additions. This may in turn lead to pores/voids in the bulk of the material [28] and compromise the quality of a root canal treatment. Hence, in this study we tested the effect of CHX on radiopacity of sealers to investigate the magnitude of correlation with solubility. To enable this, both elemental analysis of the sealer surfaces and their leachates was performed indicating the elution of radiopacifiers.

SEM analysis provided detailed information on the elemental constitution (EDS) of the sealers and the microstructural characteristics of their surfaces. For leachates analysis, ICP-OES was used for testing as it is one of the most reliable methods currently available [29], and is significantly more accurate in comparison with other techniques commonly applied, namely the use of different element probes [30]. The chemical analysis of sealer leachates was coupled with the assessment of pH, which may reflect the sealers' antimicrobial activity.

Wettability, microhardness and surface roughness were also assessed in this study. Surface roughness of substrates has been related to initial bacterial adhesion in the course of biofilm formation [31]. We used stereoscopic profilometry, which is a sensitive and non-destructive technique that images an area of the surface without being in contact with the specimens. Additionally, both microscopic (SEM micrographs) and macroscopic (photography) inspection of the samples was performed for qualitative analysis of the sealers' surface characteristics. Wettability of sealers can influence their ability to adhere to dentine and penetrate the dentinal tubules, which affect indirectly the antimicrobial efficacy of sealers [16,18,32]. Microhardness assesment of a material is a measure of multiple properties. It can be used as an indicator of the setting process as well as to show how different setting conditions can affect the overall surface strength of a material [33]. Low microhardness of dental materials has been associated with reduced bond strength to dentine and sealing ability [34].

We found no effect of CHX on the solubility of the sealers. A previous study on sealers' solubility using ISO 4049 methodology reported various effect of CHX on AH Plus, Bioroot RCS and PCS [35]. A recent publication evaluating AH Plus, MTA Fillapex and PCS with incorporated CHX nanoparticles showed similar results to our study: incorporated CHX had no effect on solubility [36]. AH Plus and PCS reported low solubility values ($< 3\%$) in all immersion periods, while BioRoot RCS presented the first 24 h high solubilty ($> 15\%$) with decreasing tendency ($< 3\%$ after 7 days). Similar results about AH Plus, Pulp Canal Sealer and BioRoot RCS have been found in other studies [12,37–40]. Both AH Plus and PCS are hydrophobic materials [35], whereas BioRoot RCS is a highly hydrophilic material with greater porosity and solubility [41]. Moreover, its hydraulic nature and the formation of soluble calcium hydroxide and calcium salts, which are rapidly washed out by water, renders the sealer susceptible to the environmental conditions [25,42]. On the other hand, conventional solubility tests may overestimate the solubility of hydrophilic sealers such as calcium silicate-based sealers [43,44]: the water to sealer mass ratio is substantially higher in solubility testing than in clinical scenario [25]. This may indicate a need for revising the methodology in solubility testing of hydraulic calcium silicate materials [45].

CHX had no effect on the sealers' radiopacity and had no effect on the elution of elements with radiopacifying potential (W for AH Plus, Zr for BioRoot RCS and Zn for PCS). A recent study that modified AH Plus, MTA Fillapex and PCS with incorporated CHX nanoparticles also showed no significant effect of CHX on radiopacity [36]. Immersion in water reduced only BioRoot's radiopacity over time. This correlated with its solubility, whereas the low solubilty values of AH Plus and PCS corresponded to radiopacity over time. Both AH Plus and PCS showed similar radiopacity values (9.5 mm Al) while BioRoot RCS had lower radiopacity (from 3 mm to 4.4 mm Al). These values are similar to those reported in the literature [36,37,41].

In the present study, wettability, microhardness and surface roughness were assessed on 28 days set sealers. BioRoot in contrast with AH Plus and PCS was highly hydrophilic as complete wetting was observed in all conditions tested, in line with previous reports [15,41]. Contact with CHX rendered AH Plus more hydrophilic and immersion in water had the same effect. This may be explained by the fact that AH Plus is sensitive to moisture from residual substances derived from intracanal medications and irrigation solutions [46]. Presumably, the moisture from the aqueous CHX increased the hydrophilicity of AH Plus [15]. Contrarily, CHX increased the hydrophobicity of PCS while no effect was shown on immersed to water samples. The effect of CHX appears opposite to our previous findings [15], which may be explained by the longer aging time of the sealer in the present study (28 days vs 24 h): one may speculate that the rough surface texture of PCS is associated with water evaporation while the hydrophobic CHX molecules remain linked/attached on the sealers' surface. Thus, a more hydrophobic equilibrium may be established for PCS after CHX contact, whereas immersion in water for 28 days increased hydrophilicity of the sealer. BioRoot RCS was not affected by either CHX contact or immersion to water, affirming the highly hydrophilic nature of the sealer [25,41].

CHX did not affect the microhardness of AH Plus. On the other hand, we found that contact with CHX compromised AH Plus's microhardness after 24 h ageing in a previous study [15]. This result indicates that CHX might have an effect on the first hours of setting process. Hydraulic calcium silicate-based cements, such as BioRoot RCS, present increased adsorption of water due to high hydrophilicity of their surfaces. A study comparing the physical properties of AH Plus, PCS and two calcium silicate-based sealers, BioRoot RCS and MTA Fillapex reported higher water sorption and porosity for BioRoot RCS [41]. Moreover, a study on setting of a premixed calcium phosphate silicate–based sealer (Endo-Sequence BC Sealer, Brasseler, Savannah, GA) documented a reduction in microhardness when additional water was added to the sealer [47]. In this respect, the differences reported in our study in microhardness assays are in accordance with the setting behaviour of BioRoot RCS under CHX and water exposure. The present study showed that PCS sealer exhibited low microhardness values, which was further reduced by CHX. This is in accordance with the low compressive strength previously reported for PCS sealer [48].

Surface roughness of sealers was evaluated together with macro- and microscopical qualitative analysis of the samples. BioRoot RCS presented the highest surface roughness values followed by PCS and AH Plus. This finding is in accordance with previous studies [15,41]. As shown, BioRoot RCS is hydrophilic and exhibits high water sorption, which in turn increases porosity [35]. Two ex vivo studies found higher porosity for BioRoot RCS compared to AH Plus upon assessment with micro-computed tomography [49,50]. This was also evident in our study by the pores and the irregular texture that was observed under both macro- and microscopical evaluation. This may be of clinical relevance as open pores may lead to growth of residual bacteria [50]. Higher porosity was found mainly for non-immersed BioRoot RCS while CHX contact reduced the surface roughness. Under microscopy, discrete round crystals could be identified on the non-immersed BioRoot samples; these are probably products of the interaction with the atmospheric air and the formation of calcium carbonate [45]. This can explain the elevated surface roughness for non-immersed samples while contact or immersion to solutions lowers carbonation reactions. Moreover, the carbonation effect and the long ageing time seem to mask the effect of CHX to increase surface roughness after 24 h ageing period of BioRoot RCS [15]. Nevertheless, surface roughness of BioRoot RCS remained relatively high after immersion to water as leaching of filler particles and loss of matrix can leave a non-homogeneous rough surface [51]. AH Plus without immersion exhibited lower surface roughness than the immersed sealer and CHX had no effect, whereas in the abovementioned study CHX increased roughness of the surfaces. Again, based on these results and given the longer ageing time in our study, the early events of setting reactions of the sealers may differ in a wider timeframe, especially when placed in varying environments. PCS alone and without immersion displayed the lowest surface roughness compared to the other conditions investigated. PCS has previously exhibited pronounced shrinkage when stored at 100% humidity [52], and a zinc oxide-eugenol impression material showed a maximum reduction in dimensions after disinfection with aqueous CHX solutions [53]. PCS does not favour water absorption and exhibits low porosity [41] which coupled with shrinkage of the sealer leads to low surface roughness [15]. However, in our study long-term contact with water led to extensive shrinkage, induced cracks in the bulk of the material and to high surface roughness values. CHX in contact with non-immersed PCS increased the roughness of the sealer over time, indicating a long-term time effect on the sealer, whereas this is not observed in the early stages of setting [15]. Differences in surface roughness between studies for the same three sealers using mechanical and optical profilometry may be attributed to the different methodological characteristics of the techniques.

When CHX was applied to AH Plus and PCS surfaces they exhibited peaks of chlorine originating from CHX. This may indicate cross-linking of CHX to sealers' surfaces [15]. CHX deposition on BioRoot RCS could not be monitored by tracing chlorine as the sealer contains calcium chloride [41]. While unset materials can potentially leach more compounds [54], for PCS, zinc showed a constant/gradual release of the element over time. Overall, CHX did not affect the concentration of most elements both in short- and long-term elution as well as the pH values of the sealers. The concentration of elements that were released by AH Plus (Ca, Zr, W, Si), especially Ca, were substantially lower than in BioRoot RCS, which as a hydraulic cement releases calcium/hydroxyl ions when in contact with water, resulting in increasing alkalinity [25,36]. These findings corroborate previous studies on elemental analysis and pH assessment of the sealers [25,51]. AH Plus has good long-term dimensional stability and low solubility [1,51,55,56], which may also explain its chemical stability reported in our study earlier [57,58].

There is scant scientific data about the potential interactions between endodontic sealers and irrigation solutions. The present study within its limitations contributes to a greater understanding and knowledge about the effect of CHX. Future efforts should include the evaluation of other irrigation solutions that are suggested for use as last irrigants before sealer placement in the root canal system such as EDTA and sodium hypochlorite. Correlations between sealers' physicochemical performance and their biological properties (antimicrobial activity and cytotoxicity) may be of clinical relevance. Customisation of the techniques and materials used in endodontic treatments would ensure that root canal fillings as a whole maintain their biological properties over time without compromising their physicochemical performance.

5. Conclusions

CHX affected sealers' physicochemical properties to various extents. Exposure to CHX did not affect solubility and radiopacity of the sealers, while wettability, microhardness and surface roughness were altered. AH Plus and PCS surfaces exhibited chlorine peaks after contact with CHX suggesting retention of CHX on their surface, whilst sealer leachates were not affected in terms of elements' concentration and alkalinity.

Declaration of Competing Interest

none.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dental.2023.11.019.

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