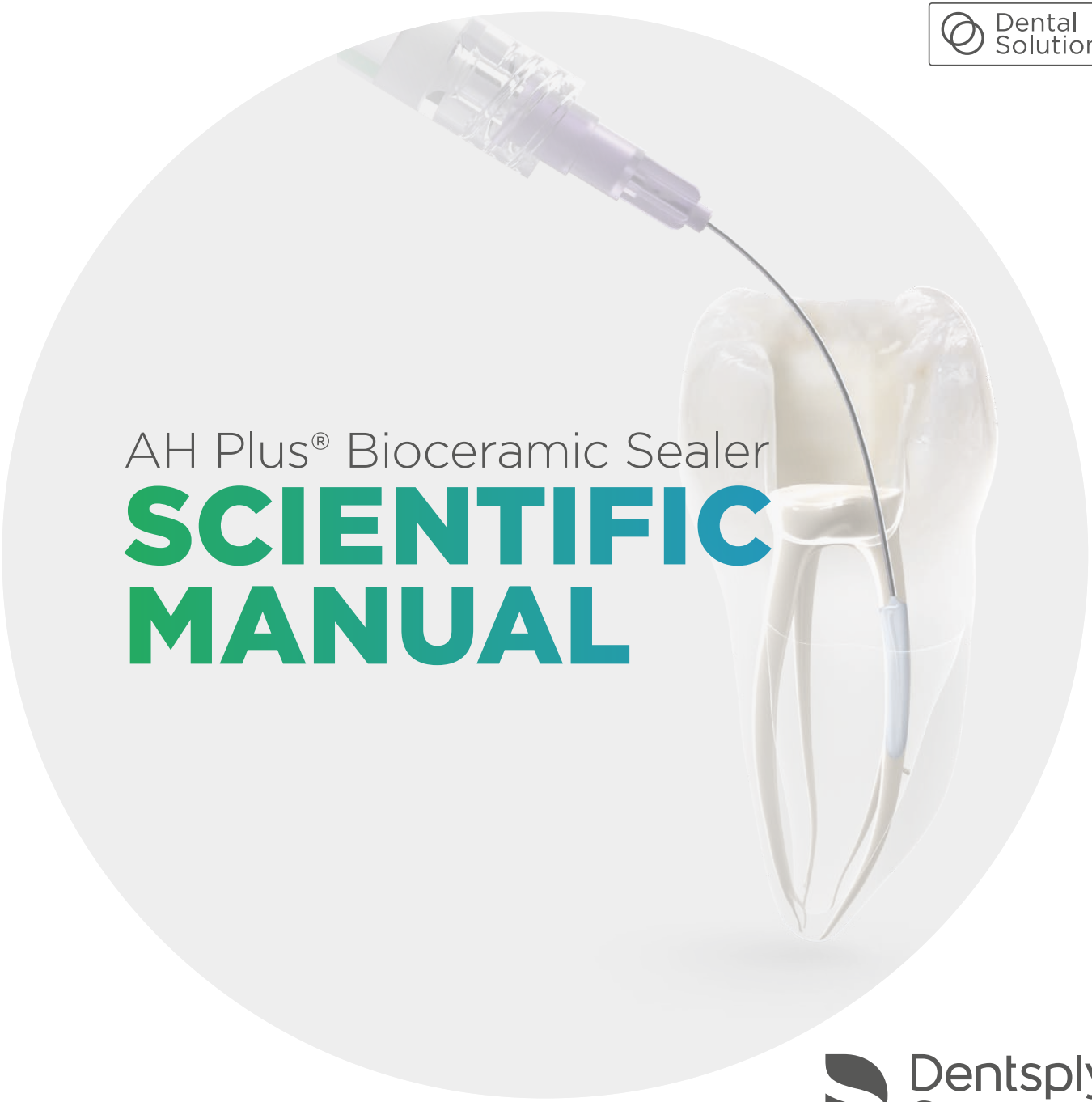
Dental Solution



AH Plus® Bioceramic Sealer

SCIENTIFIC MANUAL

dentsplysirona.com/AHPlusBioceramicSealer

Dentsply Sirona

AH Plus® Bioceramic Sealer

Table of contents

AH Plus® Bioceramic Sealer – description

AH Plus® Bioceramic Sealer is the latest sealer in the AH Plus® product family. It is a calcium silicate-based sealer, ready-to-use, with pre-loaded material confined into an air-tight syringe that permits its direct application into the root canals (*Figure 1*). During injection, AH Plus® Bioceramic Sealer absorbs the environmental moisture as basis for the hydration reaction and sets without the need of previous powder/liquid or base/catalyst mixing.

AH Plus® Bioceramic Sealer is derived from Mineral Trioxide Aggregate (MTA). MTA has been widely used in a variety of applications in endodontics as supported by a broad body of evidence. MTA has excellent physical and biological properties such as biocompatibility, bioactivity, and sealing ability[1-3]. This sealing capacity is largely attributed to MTA's bioactivity and ability to release calcium ions and produce an apatite layer in the presence of phosphate-containing physiological fluids[4-6].

AH Plus® Bioceramic Sealer contains:

- 5 - 15 wt% Tricalcium Silicate as core material and responsible for setting of the sealer,
- Zirconium Dioxide as radiopacifier,
- Dimethyl Sulfoxide (DMSO) as non-reactive diluent to convey suitable flow properties of the paste,
- Lithium Carbonate to support the setting,
- and other ingredients, bentonite clay, polyvinyl alcohol and polyvinyl pyrrolidone, as additional components.

The hydration reaction of Tricalcium Silicate leads to the formation of Calcium Silicate Hydrate and Calcium Hydroxide.* Calcium Hydroxide increases the pH, which induces the formation of hydroxyapatite (HA) (see ***Figure 2*** showing HA crystals in in vitro conditions and ***Figure 3*** showing HA peaks in AH Plus® Bioceramic sealer via Raman spectroscopy:

$Ca_3SiO_5 + 5.2H_2O \rightarrow 1.2Ca(OH)_2$ (Calcium Hydroxide)+ $(CaO)_{1.8}SiO_2(H_2O)_{4.0}$ (Calcium Silicate Hydrate)

Cured sealer-samples were set in a humidity chamber at 37°C for 1 week then were immersed in 2 ml simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma.

Its basic physico-chemical properties are summarized in ***Table 1***, in comparison with AH Plus® sealer and EndoSequence BC sealer (Brasseler).

* Cuesta A, et al. Multiscale understanding of tricalcium silicate hydration reactions. Sci Rep. 2018 Jun 4;8(1):8544.

Figure 1:
AH Plus® Bioceramic Sealer.

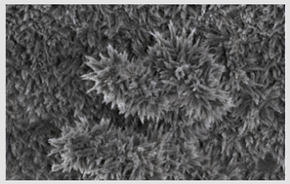


Figure 2:
Hydroxyapatite crystals on samples of AH Plus Bioceramic Sealer using High-resolution Scanning Electron Microscope (HR-SEM; Hitachi SU8000) (internal data, on file).

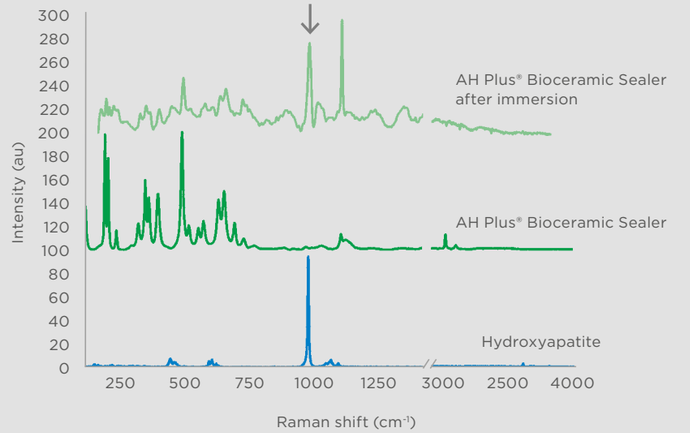


Figure 3:
*Hydroxyapatite (HA) detection using Raman Spectroscopy (scattering technique), distinctive peaks (arrow) of hydroxyapatite (reference HA material from Sigma-Aldrich). Raman spectroscopy measurements were performed at room temperature using a Horiba LabRam Raman microscope equipped with Leica microscope, a CDD camera, and laser at 532 nm with 10 mW power.**

Table 1:
*Material properties according to ISO 6876 of AH Plus® Bioceramic Sealer in comparison to other sealers.**

Basic material properties				
Tests	**Requirements**	**AH Plus® Bioceramic Sealer**	**Brassler's EndoSequence BC Sealer**	**AH Plus®**
Radiopacity (mmAl)	≥ 3 mmAl/mm	7.5 mmAl/mm	6.3 mmAl/mm	9.2 mmAl/mm
Flow (mm)	≥ 17 mm	26 mm	22 mm	24 mm
Set Time (hours)	As stated in IFU (within 10%)	2 - 4 hours	4 – 10 hours	24 - 48 hours
Film thickness (µm)	≤ 50 µm	25 µm	70 µm	25 µm
Solubility	≤ 3%	0.11%	23.00%	0.22%

* Internal data on file. For more information, contact Consumables-Data-Requests@dentsplysirona.com.

In vitro/ex vivo studies

AH Plus® Bioceramic Sealer was first launched in 2021. This scientific manual provides a synopsis of the key published research findings on AH Plus® Bioceramic Sealer with focus on its physico-chemical properties and biological behavior. Each summary is based on facts retrieved from the original research article.

For an easier comparison, the outcomes of all studies included in the present scientific manual are summarized in ***Table 2***, below.

*Tests on solubility and setting time revealed various outcomes among literature, making it difficult to compare studies with each other. Some methodological variations might explain the different outcomes, making the comparison within a study valid, but comparison with other studies may not be possible. See also the publication of Camilleri *et al.*[14] for further explanation on the setting times and solubility data discrepancies. "=" means no statistically significant differences ">" and "<" indicate statistically significant differences. Total Fill BC, Essenseal, BC sealer, Ceraseal, NeoSealer Flo, Well Root ST, PCS are not registered trademarks of Dentsply Sirona Inc.

Manufacturers: Well-Root ST (Vericom, Gangwon-Do, Korea), Ceraseal (Meta Biomed), NeoSealer Flo (Avalon Biomed), Total Fill BC sealer (FKG Dentaire), Pulp Canal Sealer EWT (PSC) (Kerr Corporation) and EssenSeal (Produits Dentaires SA).

Table 2:
Summary and comparison of study outcomes included in the scientific manual.

	Donnermeyer et al.[7]	Gaeta et al.[8]	Chaves et al.[9]	Bilvinaite et al.[10]	Sanz et al.[11]	Zamparini et al.[12]	Kharouf et al.[13]
pH	AH Plus Bio = Total Fill BC (alkaline pH)> AH Plus (neutral pH)	NA	AH Plus Bio = BC sealer (alkaline pH)> AH Plus (neutral pH)	NA	NA	Ceraseal = AH Plus Bio = NeoSealer Flo (alkaline pH) > AH Plus (neutral pH)	AH Plus Bio = Well Root ST (alkaline pH) > AH Plus (neutral pH)
Flow	AH Plus Bio = Total Fill BC = AH Plus (all ISO 6876 compliant)	PCS > Essenseal = AH Plus Bio (all ISO 6876 compliant)	AH Plus Bio = BC sealer = AH Plus (all ISO 6876 compliant)	NA	NA	AH Plus = Ceraseal > AH Plus Bio > NeoSealer Flo (all ISO 6876 compliant)	NA
Radiopacity	NA	AH Plus Bio = Essenseal > PCS (all ISO 6876 compliant)	AH Plus > AH Plus Bio > BC sealer (all ISO 6876 compliant)	AH Plus Bio highest radiopacity vs. all evaluated sealers	NA	AH Plus > AH Plus Bio > Ceraseal > NeoSealer Flo (all ISO 6876 compliant)	NA
Film Thickness	All ISO 6876 compliant (slight modifications of the test protocol due to preheating of the sealers)	AH Plus Bio > Essenseal = PCS (all ISO 6876 compliant)	NA	NA	NA	AH Plus Bio > NeoSealer Flo > Ceraseal = AH Plus (all ISO 6876 compliant)	NA
Compression strength	NA	NA	NA	NA	NA	NA	AH Plus Bio < Well Root ST = AH Plus
Roughness	NA	NA	NA	NA	NA	NA	AH Plus Bio = Well Root ST > AH Plus
Wettability & porosity	NA	NA	NA	NA	NA	NA	AH Plus Bio = Well Root ST > AH Plus
Cell viability	NA	NA	AH Plus Bio = BC sealer > AH Plus	NA	AH Plus Bio = BC sealer > AH Plus		AH Plus Bio = Well Root ST > AH Plus
Mineralization	NA	NA	Calcium release: BC sealer > AH Plus Bio > AH Plus	NA	Cell mineralization: BC sealer > AH Plus Bio > AH Plus	Calcium release: Ceraseal > AH Plus Bio = NeoSealer Flo > AH Plus	NA
Setting time*	AH Plus < AH Plus Bio <Total Fill BC. All compliant with ISO 6876 according to the authors	Essenseal < AH Plus Bio < PCS	AH Plus < AH Plus Bio < BC sealer	NA	NA	AH Plus Bio < Ceraseal < AH Plus < NeoSealer Flo	NA
Solubility* (wt%)	AH Plus Bio = Total Fill BC > AH Plus. (AH Plus Bio and Total Fill above ISO 6876 threshold)	AH Plus Bio = Essenseal = PCS. (All compliant with ISO 6876)	AH Plus Bio = BC sealer > AH Plus. (AH Plus Bio and Total Fill above ISO 6876 threshold)	NA	NA	NeoSealer Flo > AH Plus Bio > Ceraseal >AH Plus. (AH Plus Bio and NeoSealer above ISO 6876 threshold)	NA

1. Physico-chemical properties

Zamparini F, Prati C, Taddei P, Spinelli A, Di Foggia M, Gandolfi MG. Chemical-Physical Properties and Bioactivity of New Premixed Calcium Silicate-Bioceramic Root Canal Sealers. Int J Mol Sci. 2022 Nov 11;23(22):13914[12].

Physico-chemical properties and apatite formation ability of AH Plus® Bioceramic Sealer was compared in vitro with two other calcium silicate-based sealers Ceraseal (Meta Biomed) and NeoSealer® Flo (Avalon Biomed), and with AH Plus® resin-based sealer, as control.

Regarding physico-chemical properties, setting times, radiopacity, solubility, water absorption, open porosity (i.e. linked to the ability of the sealer to absorb water and release ions), flow and film thickness were evaluated, along with the calcium release ability of sealers.

The outcomes are the following (see *Table 3*):

- AH Plus® Bioceramic Sealer is significantly more radiopaque than other evaluated calcium silicate-based sealers, but less than AH Plus®.
- All evaluated sealers complied with ISO 6876 regarding their flowability and film thickness evaluations.
- The pH was alkaline for the three evaluated calcium silicate-based sealers and at all time periods, decreasing with time. On the other hand, AH Plus® resin-based sealer shows a slightly decreasing pH over the same period of time, in the neutral pH range.
- All evaluated calcium silicate-based sealers released calcium after soaking in water, from 3h to 28 days (see ***Table 4***).

Table 3:
Alkalizing activity (mean ± SD; n = 8) of tested materials. The pH of soaking water was measured after immersion of set sealers (+100% of final setting time). Different superscript letters (vertical row) indicate statistically significant differences (p < 0.05) among sealers.

	3 h	1 day	3 days	7 days	14 days	28 days
Ceraseal	9.51 ± 0.10 [a]	10.01 ± 0.28 [a]	9.64 ± 0.34 [a]	9.01 ± 0.13 [a]	8.75 ± 0.15 [a]	8.13 ± 0.16 [a]
NeoSealer Flo	8.73 ± 0.10 [b]	8.40 ± 0.04 [b]	8.23 ± 0.12 [b]	8.43 ± 0.03 [b]	8.38 ± 0.06 [b]	8.17 ± 0.11 [a]
AH Plus Bioceramic	8.6 ± 0.6 [b]	9.5 ± 0.5 [ab]	9.0 ± 0.6 [a]	8.4 ± 0.5 [b]	8.4 ± 0.5 [b]	8.3 ± 0.6 [a]
AH Plus	7.7 ± 0.2 [c]	7.4 ± 0.1 [c]	7.3 ± 0.2 [c]	7.3 ± 0.2 [c]	7.2 ± 0.1 [c]	7.1 ± 0.3 [c]
Deionized water	7.02 ± 0.17 [d]	7.28 ± 0.32 [c]	7.12 ± 0.32 [c]	7.05 ± 0.35 [c]	7.12 ± 0.32 [c]	6.98 ± 0.25 [c]

Table 4:
Calcium release (ppm, mean ± SD; n = 8) of tested sealers. Calcium release in soaking water was measured after immersion of set materials (+100% of final setting time). Different letters indicate statistically significant differences (p < 0.05) among materials.

	3 h	1 day	3 days	7 days	14 days	28 days	Cumulative
Ceraseal	43.73 ± 5.7 [a]	80.85 ± 10.89 [a]	89.52 ± 5.68 [a]	57.53 ± 10.31 [a]	41.95 ± 4.65 [a]	38.30 ± 13.18 [a]	347.45 ± 16.25 [a]
NeoSealer Flo	40.56 ± 14.4 [a]	41.37 ± 8.6 [b]	47.95 ± 19.9 [b]	36.77 ± 7.2 [b]	27.25 ± 2.4 [b]	21.77 ± 2.4 [b]	205.25 ± 35.4 [b]
AH Plus Bioceramic	20.5 ± 10.6 [b]	30.6 ± 10.5 [b]	67.5 ± 20.5 [c]	40.5 ± 7.5 [b]	35.5 ± 10.5 [ac]	20.58 ± 8.6 [b]	200.5 ± 80.5 [b]
AH Plus	1.8 ± 0.8 [c]	1.9 ± 0.5 [c]	1.3 ± 0.4 [d]	2.1 ± 0.2 [c]	2.5 ± 1.2 [d]	0.5 ± 0.2 [c]	1.4 ± 0.5 [c]
Deionized water	1.6 ± 0.5 [c]	1.5 ± 0.3 [c]	1.2 ± 0.1 [d]	1.1 ± 0.1 [c]	1.5 ± 0.6 [d]	0.5 ± 0.2 [c]	0.7 ± 0.5 [c]

Regarding setting time and solubility, the outcomes were:

- AH Plus® Bioceramic Sealer has similar final setting time compared to Ceraseal in 720 +/- 60 min. NeoSealer Flo and AH Plus show the longest final setting time (significant difference with other evaluated sealers) above 1300 min.
- Solubility of NeoSealer Flo and AH Plus® Bioceramic sealers, 7.1% and 5.8% respectively, was significantly higher than the one of AH Plus® and Ceraseal, 0.8% and 1.02% respectively.

Note: Some methodological variations between the evaluated studies might explain the different outcomes. While, the comparison within a study is valid, the direct comparison with other studies may not be possible. See also the publication of Camilleri *et al.*[14] for further explanation on the setting times and solubility data discrepancies.

Kharouf N, Sauro S, Eid A, Zghal J, Jmal H, Seck A, Macaluso V, Addiego F, Inchingolo F, Affolter-Zbaraszczuk C, Meyer F, Haikel Y, Mancino D. Physicochemical and Mechanical Properties of Premixed Calcium Silicate and Resin Sealers. J Funct Biomater. 2022 Dec 23;14(1):9[13].

Physico-chemical properties and biocompatibility performance of AH Plus® Bioceramic Sealer was compared in vitro with Well-Root ST (Vericom, Gangwon-Do, Korea – Calcium silicate-based sealer) and AH Plus® resin-based sealer.

Physico-chemical properties, pH, porosity, wettability, compression strength, roughness were evaluated; along with periodontal ligament stem cell attachment on sealer's surfaces for the biological part. All sealers were injected in Teflon molds and then stored at 37°C for 48 h for setting.

The outcomes are the following:

- Both calcium silicate-based sealers have basic/alkaline pH, around 11 in distilled water, from 3h to 72 h of immersion. On the other hand, AH Plus® resin-based sealer shows a decreasing pH over the same period of time, from 8.8 to 7.3.
- Both calcium silicate-based sealers show higher porosity and wettability/hydrophilicity compared to AH Plus sealer contact angles of 0° (AH Plus Bio), 10.4° (Well-Root ST) and 64.9° (AH Plus), which is expected to enhance their adhesion to dentin.
- The surface roughness was higher for AH Plus and Well-Root ST compared to AH Plus® Bioceramic Sealer. This is attributed to the different composition and filler sizes of the sealers.
- The compression strength was the highest for AH Plus (85 MPa) followed by Well-Root ST (50 MPa) and AH Plus® Bioceramic Sealer (3 MPa). A lower compression strength could be beneficial for retreatment procedures also facilitating the sealer removal.
- After 8 days of incubation, cell attachment and morphology were assessed via Scanning Electron Microscopy (SEM), revealing thicker and more elongated cells at the surface of both calcium silicate-based sealers (i.e. signs of a good cell affinity) compared to those observed in contact with AH Plus®.

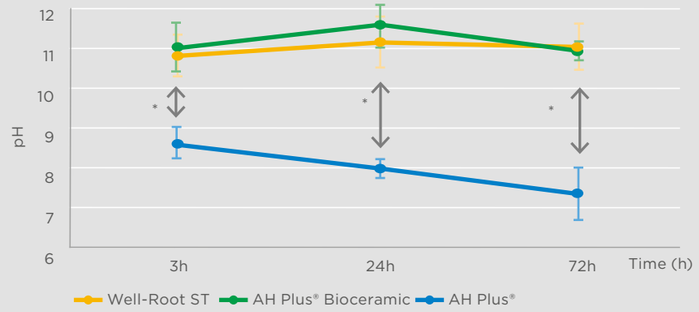


Figure 4:
pH changes with time (3, 24 and 72 h) for water in contact with AH Plus®, AH Plus® Bioceramic and Well-Root ST at 37°C. (p < 0.05).*

Donnermeyer, D.; Schemkämper, P.; Bürklein, S.; Schäfer, E. Short and Long-Term Solubility, Alkalizing Effect, and Thermal Persistence of Premixed Calcium Silicate-Based Sealers: AH Plus Bioceramic Sealer vs. Total Fill BC Sealer. Materials 2022, 15, 7320[7].

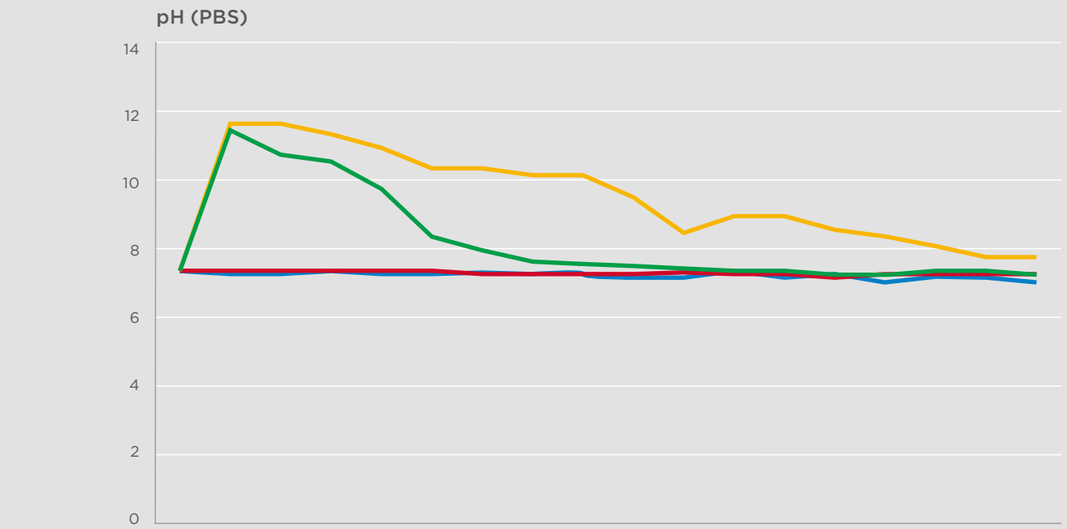
Physico-chemical properties of AH Plus® Bioceramic Sealer were compared in vitro with Total Fill BC sealer (FKG Dentaire – Calcium silicate-based sealer) and AH Plus® resin-based sealer. The solubility was evaluated in both distilled water, over one month, and in phosphate buffered saline solution, over 4 months. The pH of the solution was measured weekly (see ***Table 5***), before renewal of the solutions. Thermal stability was investigated via setting time, film thickness, and chemical analysis, after thermal treatment up to 97°C for 180 s in order to simulate warm vertical filling technique.

Both calcium silicate-based sealers have basic/alkaline pH, up to 12 in distilled water after 24 h of immersion and then somewhat decreasing to about 10 after 4 months. Their pH values are slightly lower in PBS than in distilled water, yet still in the alkaline range (see ***Figures 5***). On the other hand, AH Plus® resin-based sealer is steady around 8 over the same period of time. This alkaline/basic pH of calcium silicate-based sealers is linked to hydroxyapatite formation on their surface, that could play an important role in biocompatibility of the sealer; an alkaline/basic pH is also known to be associated with antibacterial properties.

Regarding their thermal stability, all investigated sealers did not show any chemical changes upon heating. Their setting time, film thickness and flow were not significantly affected (see ***Table 6*** below, for AH Plus Bioceramic sealer). All together, these outcomes confirmed that AH Plus® Bioceramic Sealer is compatible with warm vertical compaction techniques.

Table 5:
Means and standard deviations: solubility of AH Plus Bioceramic Sealer, Total Fill BC Sealer, and AH Plus in Distilled Water (AD) over 28 days and in Phosphate Buffered Saline solution (PBS) over 4 months, respectively. Superscript letters indicate statistically significant differences at measurement dates in AD and PBS (p < 0.05).

	AD			PBS		
Solubility in %	**AH Plus Bioceramic Sealer**	**Total Fill BC Sealer**	**AH Plus**	**AH Plus Bioceramic Sealer**	**Total Fill BC Sealer**	**AH Plus**
14 days	30.44 ± 1.00 [a]	32.75 ± 5.26 [a]	0.55 ± 0.17 [b]	19.24 ± 2.56 [a]	14.05 ± 2.35 [b]	0.02 ± 0.23 [c]
28 days	33.09 ± 0.81 [b]	35.55 ± 1.35 [a]	0.48 ± 0.20 [c]	20.80 ± 2.01 [a]	20.64 ± 2.87 [a]	0.28 ± 0.16 [b]
2 months				16.82 ± 2.38 [a]	14.78 ± 4.02 [a]	0.30 ± 0.18 [b]
4 months				18.40 ± 1.91 [a]	20.50 ± 9.23 [a]	0.32 ± 0.08 [b]



	0h	24h	7d	14d	21d	28d			2mo			3mo				4mo
AH Plus Bioceramic	7.44	11.51	10.81	10.67	9.86	8.42	8.06	7.74	7.61	7.55	7.47	7.46	7.41	7.33	7.30	7.44	7.41	7.34
Total Fill BC	7.44	11.68	11.68	11.44	11.06	10.51	10.44	10.25	10.23	9.60	8.56	8.95	9.08	8.71	8.51	8.19	7.91	7.78
AH Plus	7.44	7.39	7.40	7.44	7.41	7.36	7.36	7.36	7.33	7.32	7.26	7.38	7.24	7.33	7.22	7.29	7.29	7.22
Control	7.44	7.42	7.45	7.42	7.44	7.44	7.36	7.38	7.37	7.37	7.35	7.35	7.35	7.28	7.29	7.34	7.33	7.32

Figure 5:
pH of AH Plus Bioceramic Sealer, Total Fill BC Sealer, and AH Plus in distilled water (AD) over 28 days and in PBS over 4 months, respectively.

Table 6:
Physical properties in accordance with ISO 6876 of AH Plus Bioceramic (means and standard deviations (SD)) after thermal treatment. Statistical analysis of setting time, film thickness, and flow for AH Plus Bioceramic was performed by Kruskal–Wallis test ($p < 0.05$).

°C	Group Number	Setting Time (h)			Film Thickness (m)			Flow (mm)		
		Mean	SD	Different from Group Number	Mean	SD	Different from Group Number	Mean	SD	Different from Group Number
37 (30 s)	**1**	9.861	0.369		0.016	0.007		25.7	1.0	5
57 (30 s)	**2**	10.472	0.243		0.020	0.011		25.5	2.1	
67 (30 s)	**3**	11.156	0.184	5	0.022	0.008		22.8	0.7	
97 (30 s)	**4**	10.850	0.200		0.015	0.002		18.6	0.5	
97 (180 s)	**5**	9.200	0.225	3	0.017	0.005		16.1	0.4	1

AH Plus® Bioceramic Sealer and Total Fill BC sealer showed higher solubility, up to about 30% in both distilled water and PBS, when compared to AH Plus resin-based sealer (less than 1%)

Note: Some methodological variations between the evaluated studies might explain the different outcomes. While, the comparison within a study is valid, the direct comparison with other studies may not be possible. See also the publication of Camilleri *et al.*[14] for further explanation on the solubility data discrepancies.

Gaeta C, Marruganti C, Mignosa E, Malvicini G, Verniani G, Tonini R, Grandini S. Comparison of physico-chemical properties of zinc oxide eugenol cement and a bioceramic sealer. Aust Endod J. 2022 Nov 21.

Physico-chemical properties of AH Plus Bioceramic sealer were compared in vitro with two other calcium silicate-based sealers, Pulp Canal Sealer EWT (Kerr Corporation) and EssenSeal (Produits Dentaires SA). Following ISO 6876 requirements, flowability, solubility, film thickness, radiopacity and setting time have been evaluated. Surface of each sealer was analyzed via Scanning Electron Microscopy (SEM).

All outcomes are summarized in the following table. The three evaluated sealers fulfilled ISO 6876 standard requirements, except those on setting time where AH Plus Bioceramic sealer was measured to be 10% longer than the one indicated in ***Table 7*** but still shorter than the one of Pulp Canal Sealer EWT (Kerr Corporation).

Note: Some methodological variations between the evaluated studies might explain the different outcomes. While, the comparison within a study is valid, the direct comparison with other studies may not be possible. See also the publication of Camilleri et al.[14] for further explanation on the setting times and solubility data discrepancies.

At the surface of both Pulp Canal Sealer EWT (Kerr Corporation) and EssenSeal (Produits Dentaires SA) sealers, well spread round shaped particles embedded in matrix were observed, via Scanning Electron microscopy. AH Plus Bioceramic sealer revealed a uniform surface with globular shaped structures.

Table 7:
Physico-chemical properties of the tested sealers. The setting time is more likely in minutes than seconds.

	AH Plus Bio	Pulp Canal Sealer EWT	EssenSeal
Flow (mm)**	25.46 ± 0.99	30.10 ± 2.17 [a]	27.64 ± 0.85
Solubility (%)*	0.137 ± 0.006	0.120 ± 0.003	0.114 ± 0.003 [a]
Setting time (s)**	**408** ± 15.37 [B]	590 ± 7.90 [ac]	119.8 ± 0.78 [ab]
Radiopacity (mmAl)*	9.83 ± 1.28	5.92 ± 2.49 [a]	9.52 ± 0.89 [b]
Film thickness (µm)**	10.24 ± 0.11 [b]	9.8 ± 0.07 [a]	10.78 ± 0.13 [ab]

Note: Values are mean ± standard deviation. Values followed by different superscript letters in each row differ significantly. One-way ANOVA test between sealers: *p < 0.05, **p < 0.001.

Chaves de Souza L, Teixeira Neves GS, Kirkpatrick T, Letra A, Silva R. Physico-chemical and biological properties of AH Plus Bioceramic. J Endod. 2022 Oct 21[9].

Physico-chemical properties and biocompatibility performance of AH Plus® Bioceramic Sealer was compared in vitro with EndoSequence BC sealer (Brasseler – Calcium silicate-based sealer) and AH Plus® resin-based sealer as control.

Regarding physico-chemical properties, setting times, radiopacity, solubility, surface characterization, pH and flow were evaluated; along with the cytotoxicity of sealers and their influence on bacteria reduction for the biological part.

The outcomes are the following (see also the *Figures 6&7*):

- AH Plus® Bioceramic Sealer is significantly more radiopaque than EndoSequence BC sealer but less than AH Plus®.
- The pH was alkaline for all evaluated sealers and at all time periods, decreasing with time, with significantly higher value for both AH Plus® Bioceramic and EndoSequence BC sealer compared to AH Plus®.
- All evaluated sealers have a flow higher than 17 mm, complying with ISO 6876 standard.
- After 3 days of incubation at 37°C and 95% humidity, surface characterization via Scanning Electron Microscopy (SEM) revealed the crystalline structure of both calcium silicate-based sealers, while the surface of AH Plus® appeared more homogeneous with embedded particles. An increasing amount of phosphorus and calcium at the surface of both AH Plus® Bioceramic and EndoSequence BC sealers was evaluated via Energy Dispersive X-Ray Spectroscopy (EDS), confirming their bioactive behavior.
- All evaluated sealers fully eliminate E.faecalis after 24h of incubation in direct contact. This can be attributed to the Alkaline pH of both AH Plus® bioceramic and EndoSequence BC sealer .
- AH Plus® bioceramic and EndoSequence BC sealers both complies with ISO 10993 standard in term of cytotoxicity, showing more than 70% cell viability after 48h.

Regarding setting time and solubility, the outcomes were:

- AH Plus® Bioceramic Sealer showed the shorter initial setting time in less than 7 h (significant difference with other evaluated sealers). Its final setting time of 19.78 h was significantly shorter than EndoSequence BC sealer (23.67 h), but longer than AH Plus® with 17 h.
- Solubility of both calcium silicate-based sealers, around 11%, was significantly higher than the one of AH Plus®, below 0.1%.

Note: Some methodological variations between the evaluated studies might explain the different outcomes. While, the comparison within a study is valid, the direct comparison with other studies may not be possible. See also the publication of Camilleri et al.[14] for further explanation on the setting times and solubility data discrepancies.

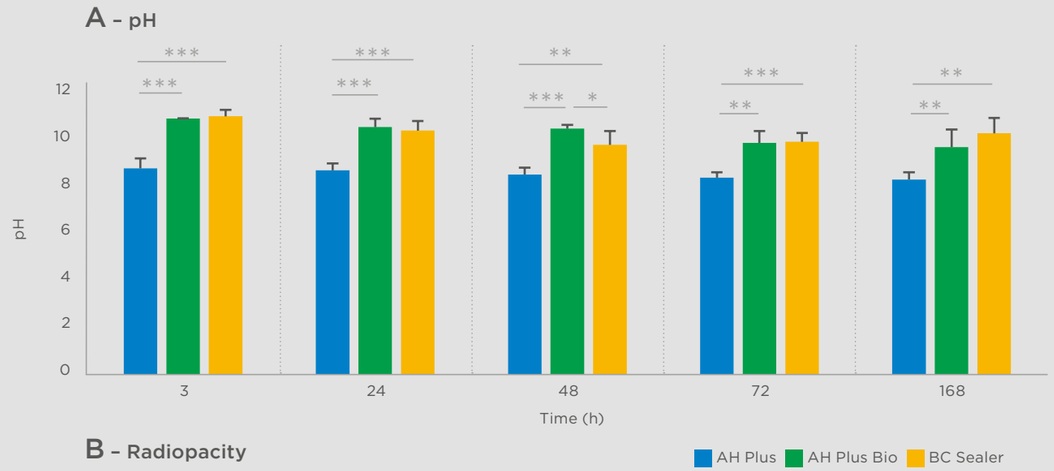


Figure 6:

*(**A**) pH.*

*(**B**) Radiopacity.*

The ISO 6876 threshold is represented by the dashed line.

** P ≤ .05*
*** P ≤ .01*
**** P ≤ .0001*
(analysis of variance and Tukey multiple comparison tests).

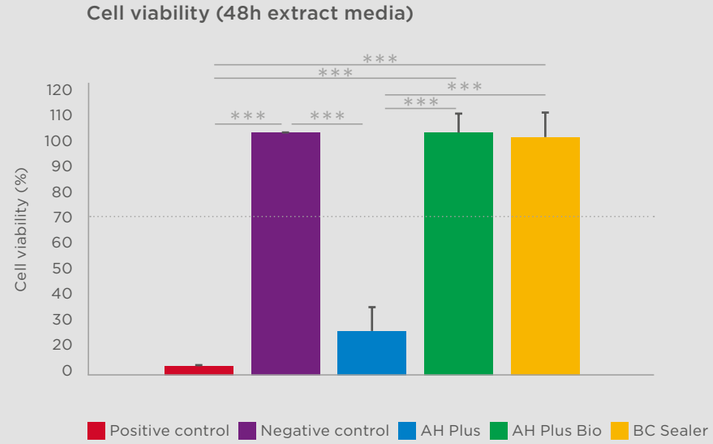


Figure 7:

Cell viability with 24-hour extract media. Sodium dodecyl sulfate was used as the positive control, and culture media was used as the negative control. The dashed line represents ISO 10993 threshold.

****P ≤ .0001 (ANOVA and Tukey multiple comparison tests).*

Bilvinaite G, Drukteinis S, Brukiene V, Rajasekharan S. Immediate and Long-Term Radiopacity and Surface Morphology of Hydraulic Calcium Silicate-Based Materials. Materials (Basel). 2022[10].

This in vitro study was dedicated to radiopacity and surface morphology comparison of AH Plus® Bioceramic Sealer (AHPB), Bio-C Sealer (BIOC), Biodentine (BD), BioRoot RCS (BR), Grey-MTAFlow (GMF), White-MTAFlow (WMF), TotalFill BC Sealer (TF), and TotalFill BC Sealer HiFlow (TFHF), 30 min after initial setting, followed by 24h and 28 days assessments.

Manufacturers: Well-Root ST (Vericom, Gangwon-Do, Korea), Ceraseal (Meta Biomed), NeoSealer Flo (Avalon Biomed), Total Fill BC sealer (FKG Dentaire), Pulp Canal Sealer EWT (PSC) (Kerr Corporation) and EssenSeal (Produits Dentaires SA).

Table 8:
Mean values and standard deviations of radiopacity at different time moments.

	Radiopacity (mm Al)			
Material	30 min	24 h	28 days	Total Increase
AHPB	10.82 ± 0.69	11.07 ± 0.94	11.26 ± 0.65	0.44
BIOC	8.15 ± 0.44	8.17 ± 0.41	8.85 ± 0.44	0.70
BD	3.34 ± 0.43	3.35 ± 0.40	3.75 ± 0.36	0.41
BR	7.19 ± 0.32 [a]	7.47 ± 0.35 [b]	8.08 ± 0.40 [a,b]	0.89
GMF	6.27 ± 0.41	6.62 ± 0.37	6.98 ± 0.32	0.71
WMF	5.76 ± 0.20	5.80 ± 0.48	6.20 ± 0.46	0.44
TF	8.56 ± 0.47 [c]	9.03 ± 0.25	9.66 ± 0.73 [c]	1.10
TFHF	8.81 ± 0.30 [d]	9.19 ± 0.31 [e]	9.79 ± 0.43 [d,e]	0.98

The same superscript letter in the line indicates statistically significant differences between radiopacity values ($p < 0.05$).

All tested hydraulic sealers showed an increased radiopacity over time, all exceeding the ISO 6876 threshold of 3 mm Al. AH Plus® Bioceramic sealer was the most radiopaque (see ***Table 8*** above).

The Scanning Electron Microscopy revealed that all evaluated sealers show precipitate nucleation and growth at their surfaces, leading to a more compact and stable surface structure after 28 days of incubation in Hank's balances Salt Solution at 37°C.

2. Biological properties

Sanz JL, López-García S, Rodríguez-Lozano FJ, Melo M, Lozano A, Llena C, Forner L. Cytocompatibility and bioactive potential of AH Plus Bioceramic Sealer: An in vitro study. Int Endod J. 2022 Oct;55(10):1066-1080[11].

Biocompatibility and bioactivity behavior of AH Plus® Bioceramic Sealer was compared in vitro with EndoSequence BC sealer (Brasseler – Calcium silicate-based sealer) and AH Plus® resin-based sealer. The influence of the sealers was investigated qualitatively and quantitatively (i) on human periodontal ligament stem cells viability, adhesion and proliferation (as part of the biocompatibility assessment), and (ii) on cells differentiation and mineralization (as part of the bioactivity assessment, via Alizarin Red S staining and RT-qPCR).

Adherent stretched cells were visible at the surface of AH Plus® Bioceramic Sealer and EndoSequence BC sealer, no cytotoxicity and a similar proliferation/migration of the cells to that of control group (cells in unconditioned medium) was observed (see ***Figure 8***), all together indicating a good biocompatibility of the two calcium silicate-based sealers.

Significant mineralization, supported by calcified nodule presences observed, and significant signs of cells differentiation into cementogenic, osteogenic and odontogenic pathways were observed with AH Plus® Bioceramic Sealer and EndoSequence BC sealer (higher mineralization), confirming their bioactivity and mineralization potentials.

These in vitro results support the good biocompatibility and bioactivity of AH Plus® Bioceramic sealer.

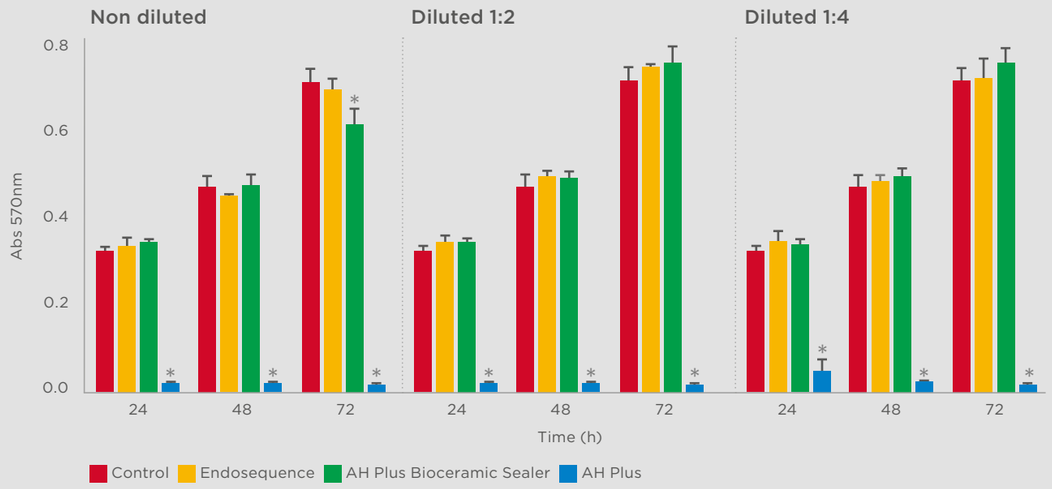


Figure 8:
Results from MTT assay for the different eluates (1:1, 1:2, 1:4) of the tested sealers (ESbcs, AHPbcs and AHP) after 24, 48 and 72 h of culture with hPDLSCs. Data are presented absorbance values (570 nm) at the different measurement time-points, compared with the negative control group. MTT colorimetric assays are used for evaluation of cellular metabolic activity (cell viability, proliferation, cytotoxicity). The assay solution color change from yellow to purple results in a higher optical density at 540 nm, which indicates a high number of viable, metabolically active cells.

** p < .001 (One-way anova analysis).*

Chaves de Souza L, Teixeira Neves GS, Kirkpatrick T, Letra A, Silva R. Physico-chemical and biological properties of AH Plus Bioceramic. J Endod. 2022 Oct 21[9].

This publication, studying both the physico-chemical properties and the biological behavior of AH Plus® Bioceramic Sealer, AH Plus® resin-based sealer, EndoSequence BC sealer (Brasseler – Calcium silicate-based sealer) is fully summarized on ***Page 15*** ("physico-chemical properties").

Its conclusions regarding biocompatibility are the following:

- All evaluated sealers fully eliminate E.faecalis after 24h of incubation in direct contact. This can be attributed to the Alkaline pH of all sealers, which is even higher for both AH Plus® bioceramic and EndoSequence BC sealer compared to AH Plus®.
- AH Plus® bioceramic and EndoSequence BC sealers both complies with ISO 10993 standard in term of cytotoxicity, showing more than 70% cell viability after 48h.

Kharouf N, Sauro S, Eid A, Zghal J, Jmal H, Seck A, Macaluso V, Addiego F, Inchingolo F, Affolter-Zbaraszczuk C, Meyer F, Haikel Y, Mancino D. Physicochemical and Mechanical Properties of Premixed Calcium Silicate and Resin Sealers. J Funct Biomater. 2022 Dec 23;14(1):9[13].

This publication, studying both the physico-chemical properties and the biological behavior of AH Plus® Bioceramic Sealer, Well-Root ST, compared to AH Plus® resin-based sealer, is fully summarized on ***Page 10*** ("physico-chemical properties").

Its conclusion regarding biocompatibility is the following:

- After 8 days of incubation, cell attachment and morphology were assessed via Scanning Electron Microscopy (SEM), revealing thicker and more elongated cells at the surface of both calcium silicate-based sealers compared to those observed in contact with AH Plus®.

Clinical aspects

AH Plus® Bioceramic Sealer can be used either alone or in combination with gutta-percha obturating cones, injected gutta-percha material or core-carriers master cones.

A clinical case with AH Plus® Bioceramic sealer is shown below in Figure 9, with a 11 months follow-up radiograph.

Figure 9:
Clinical case kindly shared by Prof. Mario Zuolo (Brazil). Maxilar central right incisor referred for endodontic treatment. Preoperative radiograph showing some type of obstruction of the entrance of the canal and apical lesion (image on the left). Shaping was done with the Reciproc system R40 and R50. The root canal was obturated with the matched-single-cone technique and AH Plus Bioceramics Sealer (image in the middle). The 11-months follow-up radiograph shows that apical healing is in progress.

Conclusion and take-home messages

Altogether, the peer-reviewed studies included in the present scientific manual allow to conclude on:

- The excellent radiopacity of AH Plus® Bioceramic sealer, superior to all other evaluated calcium silicate-based sealers and complying with ISO 6876 requirement.[8-10, 12]
- Its flowability and film thickness complying with ISO 6876 requirement.[7-9, 12]
- Its alkaline pH, linked with hydroxyapatite formation on its surface.[7, 9, 11-13]
- The compatibility of AH Plus® Bioceramic Sealer with warm obturation techniques.[7]
- AH Plus® Bioceramic sealer showed a very good biocompatibility (complying with ISO 10993 requirement), along with data suggesting bioactive and mineralization behavior.[9, 11-13]

Despite the lower tricalcium silicate content of AH Plus® Bioceramic Sealer compared to some competitive calcium silicate-based sealers, the peer-reviewed studies included above showed:

- There is a similar amount of calcium release compared to other calcium silicate-based sealers with higher tricalcium silicate content.[12]
- The Calcium-Phosphate layer formation at the sealer's surface is comparable or even higher than with other calcium silicate-based sealers with higher tricalcium silicate content.[12]
- The alkaline pH behavior over time is comparable to other calcium silicate-based sealers with higher tricalcium silicate content[7]. This alkaline pH is linked to hydroxyapatite formation on sealer's surfaces which is expected to play an important role in sealer's biocompatibility.[7]

On the other hand, other tests on solubility and setting time revealed various outcomes, making it difficult to draw any conclusions. Some methodological variations between the evaluated studies might explain the different outcomes. While, the comparison within a study is valid, the direct comparison with other studies may not be possible. See also the publication of Camilleri et al.[14] for further explanation on the setting times and solubility data discrepancies.

Finally, these first in vitro/ex vivo results support the adequate physico-chemical properties, good biocompatibility and potential bioactivity (i.e. mineralization) of AH Plus® Bioceramic sealer.

References

1. Parirokh, M. and M. Torabinejad, Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. J Endod, 2010. 36(3): p. 400-13.
2. Torabinejad, M. and M. Parirokh, Mineral trioxide aggregate: a comprehensive literature review-part II: leakage and biocompatibility investigations. J Endod, 2010. 36(2): p. 190-202.
3. Prati, C. and M.G. Gandolfi, Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dent Mater, 2015. 31(4): p. 351-70.
4. Han, L., S. Kodama, and T. Okiji, Evaluation of calcium-releasing and apatite-forming abilities of fast-setting calcium silicate-based endodontic materials. Int Endod J, 2015. 48(2): p. 124-30.
5. Tay, F.R., et al., Calcium phosphate phase transformation produced by the interaction of the portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod, 2007. 33(11): p. 1347-51.
6. Reyes-Carmona, J.F., M.S. Felippe, and W.T. Felippe, Biomineralization ability and interaction of mineral trioxide aggregate and white portland cement with dentin in a phosphate-containing fluid. J Endod, 2009. 35(5): p. 731-6.
7. Donnermeyer, D., et al., Short and Long-Term Solubility, Alkalizing Effect, and Thermal Persistence of Premixed Calcium Silicate-Based Sealers: AH Plus Bioceramic Sealer vs. Total Fill BC Sealer. Materials (Basel), 2022. 15(20).
8. Gaeta, C., et al., Comparison of physico-chemical properties of zinc oxide eugenol cement and a bioceramic sealer. Aust Endod J, 2022.
9. Chaves de Souza, L., et al., Physico-chemical and biological properties of AH Plus Bioceramic. J Endod, 2022.
10. Bilvinaite, G., et al., Immediate and Long-Term Radiopacity and Surface Morphology of Hydraulic Calcium Silicate-Based Materials. Materials (Basel), 2022. 15(19).
11. Sanz, J.L., et al., Cytocompatibility and bioactive potential of AH Plus Bioceramic Sealer: An in vitro study. International Endodontic Journal, 2022. 55(10): p. 1066-1080.
12. Zamparini, F., et al., Chemical-Physical Properties and Bioactivity of New Premixed Calcium Silicate-Bioceramic Root Canal Sealers. Int J Mol Sci, 2022. 23(22).
13. Kharouf, N., et al., Physicochemical and Mechanical Properties of Premixed Calcium Silicate and Resin Sealers. J Funct Biomater, 2022. 14(1).
14. Camilleri, J., et al., Methods for testing solubility of hydraulic calcium silicate cements for root-end filling. Sci Rep, 2022. 12(1): p. 7100.

Notes

dentsplysirona.com

MARUCHI
2-208, Medical Industry Complex Bldg.,
42-10, Taejanggongdan-gil,
Wonju-si, Gangwon-do,
26311, Replblic of Korea

EC REP

KTR Europe GmbH
Mergenthalerallee 77,
65760 Eschborn, Germany

Dentsply Sirona