



Comparison of Cytotoxicity Between Calcium Silicate and Epoxy Resin-Based Sealer in Root Canal Treatment (Systematic Review)

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Abstract

Introduction: Epoxy resin-based sealer is the gold standard sealer because of its good physical properties. However, epoxy resin sealer has the potential for cytotoxicity which can inhibit the healing process of periapical tissue. Calcium silicate-based sealer is an alternative sealer because of its more biocompatible properties. **Methods:** The study used PubMed, Google Scholar, and Science Direct databases from 2019 to 2024. The journals used were by the inclusion and exclusion criteria, then bias analysis was carried out and summarized to determine the cytotoxicity of calcium silicate and epoxy resin-based sealer. **Results:** This study successfully synthesized 19 in vitro journals with low bias results in 16 journals and 3 journals with moderate bias. In general, calcium silicate-based sealers have lower cytotoxicity than epoxy resin-based sealers with the explanation that the composition, conditions, and exposure time of the material play an important role in the biological properties of the sealer. **Conclusion:** Calcium silicate-based sealers exhibit lower cytotoxicity than epoxy resin-based sealers.

Keywords: Calcium silicate; cytotoxicity; epoxy resin; biocompatibility

Introduction

According to research by Ingle and Beveridge, as many as 58% of endodontic treatment failures are caused by imperfect obturation.¹ The purpose of obturation in the root canal is to achieve a fluid-tight seal that can prevent intracanal recontamination.² Obturation materials consist of using solid filling materials (gutta-percha) together with root canal sealers.¹ Root canal sealers have a more important function in achieving a fluid-tight seal than solid filling materials.³

The ideal requirements for a root canal sealer include providing an excellent seal when set, producing sufficient adhesion, being radiopaque, nonstaining, dimensionally stable, easy to mix and insert into the root canal, easy to remove from the root canal if necessary, insoluble in tissue fluids, bactericidal or at least discourage bacterial growth, nonirritating to periapical tissue, and having a long setting time to obtain sufficient working time.¹ Root canal sealers must be biocompatible because they are near the periapical tissues, which can affect their repair.⁴ Sealers are expected not to cause tissue damage or cell death.³



Epoxy resin-based sealer is considered the gold standard because of its good physical properties. The drawback that still exists in epoxy resin-based sealer is the possibility of cytotoxicity.⁵ The combination of the release of epoxy resin components and formaldehyde is the cause of cytotoxicity in sealer. An alternative sealer to overcome the problem of cytotoxicity of epoxy resin-based sealer is calcium silicate-based sealer.⁶

Calcium silicate-based sealers are currently widely used in endodontic practice due to their biocompatible properties.⁷ A study conducted by Dioguardi et al. (2021) found that calcium silicate sealers exhibit biological and mechanical properties comparable to epoxy resin sealers.⁸ Calcium silicate sealers have bioactive properties that can stimulate the formation of hard tissue in bone and periodontal ligaments. The results of the study confirmed the potential of calcium silicate sealers as a promising alternative in root canal treatment.^{8,9}

There are many different formulations of calcium silicate sealers that have been commercially marketed today.¹⁰ Further research and clinical studies are needed because some of the marketed products are still in the early stages of development.¹¹ Therefore, this systematic review aimed to find out the comparison of cytotoxicity between calcium silicate and epoxy resin-based sealer in root canal treatment.

Methods

This systematic review was conducted using PubMed, Google Scholar, and ScienceDirect databases, covering studies published between 2019 and 2024. The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Inclusion and exclusion criteria are presented in Table 1. The search strategy, including keywords and controlled vocabulary terms, is summarized in Table 2.

Table 1. Inclusion and exclusion criteria

Criteria	
Inclusion	Exclusion
In vitro studies	Studies performed in animals, reviews and serial report
Studies using human cell cultures	
Studies using MTT or XTT assays	
<i>Calcium silicate-based sealers</i>	
<i>Epoxy resin-based sealer</i>	
Articles published in English or Indonesian	



Table 2. Data search strategy

Inclusion Criteria	Population	Intervention	Comparison	Outcome
Keyword	Cellular model	Calcium silicate- based sealer. ¹ Epoxy resin-based sealer. ²	Cytotoxicity	Cytotoxicity
Original Word	Human periodontal ligament stem cells (hPDLSCs), Human periodontal ligament fibroblast cell (hPDLFC), Human gingival fibroblast cells, Primary human osteoblasts (hOB), Apical papillary cells (APCs)	1: Calcium silicate-based sealer; tricalcium silicate, mineral trioxide aggregate, calcium silicates cements, bioceramics sealer 2: Epoxy resin- based sealer; conventional resin-based sealer	-	Cytocompatibility
Controlled Vocabulary	Human cell, stem cell, fibroblast cell, periodontal ligament, periodontal ligament cell, fibroblast, osteoblast, osteoblast cell, cell	Dental cements, root canal sealer, sealer, endodontic sealers, endodontics, root canal treatment, root canal filling	-	Cell viability, inflammatory response, biocompatibility, cell proliferation

Risk of bias assessment

The risk of bias for each include study was assessed using the following parameters: description of cell lineage, presence of control group, description of cell culture preparation, description of sealer preparation, reproducibility of the study, and description of statistical analysis.^{10,12} Each parameter was scored ‘yes’ answer if it was clearly reported in the study and ‘no’ if it was not reported. Studies fulfilling one or two parameters were classified as having a ‘high’ risk of bias, those fulfilling three to four parameters were classified as a ‘moderate’ risk of bias, and five to six parameters were classified as a ‘low’ risk of bias.

Results

The initial database search yielded a total 928 studies identified through a predefined search strategy. After the removal of 124 duplicate records, 804 studies remained for screening. Studies were selected according to the inclusion criteria, resulting in 632 studies being discarded because they were not relevant to the research question and did not meet the criteria, leaving 48 studies to be reviewed for their full articles. Following full-text evaluation, 29 articles were excluded: 16 studies used different sample types, and 13 studies employed



cytotoxicity assessment methods other than those specified in the inclusion criteria. Consequently, a total of 19 studies were included in this systematic review.

The literature search was conducted on June 3-12, 2024. This review process was presented in Figure 1. In general, the research results from the reviewed studies concluded that calcium silicate-based sealers have a lower cytotoxic effect than epoxy resin-based sealers.¹³⁻³¹

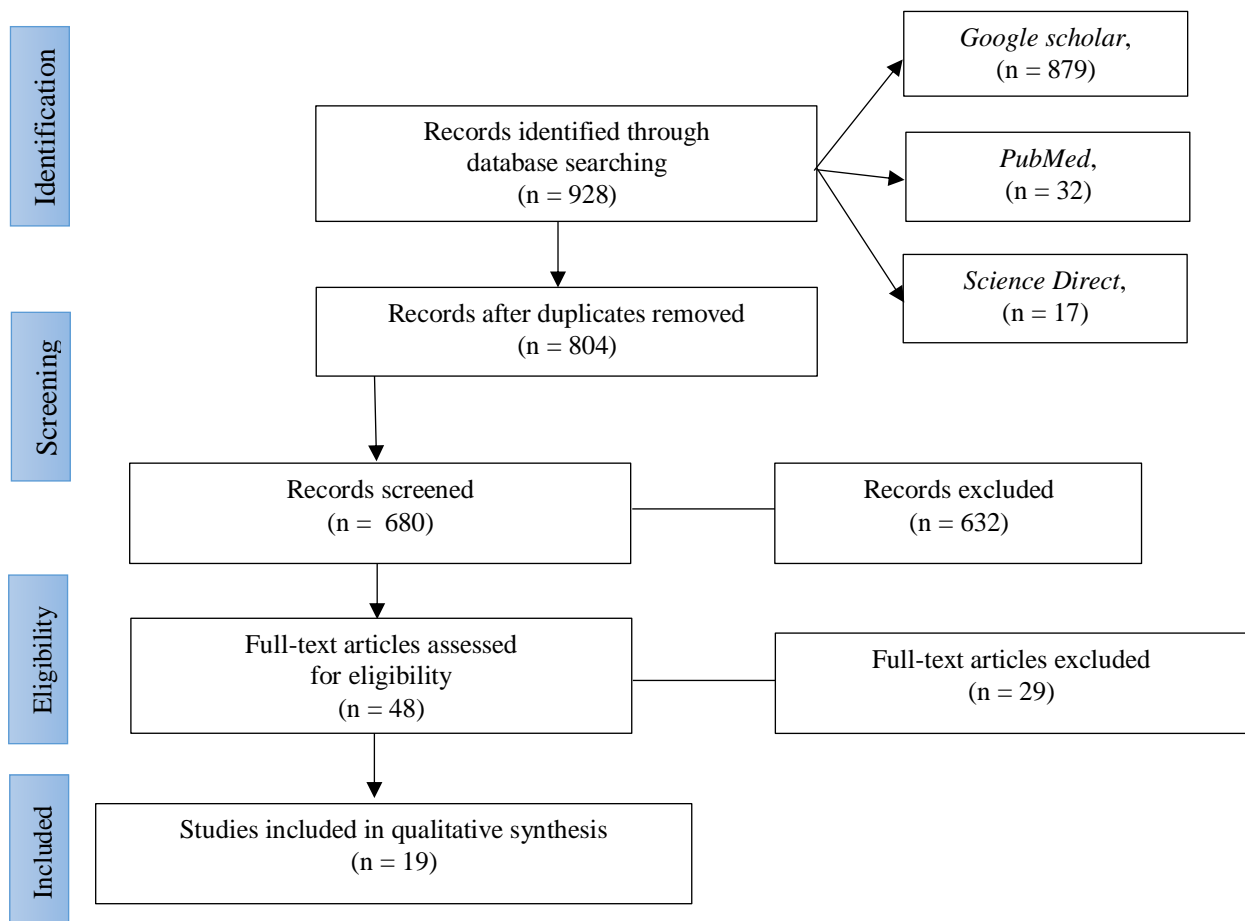


Figure 1. PRISMA flow diagram

The cytotoxicity assessment results of calcium silicate-based sealers included in this review are summarized in Table 3. The table presents the reported cell viability values and their classification according to ISO standards, providing an overview of the biocompatibility profiles of the evaluated calcium silicate-based sealers. Most calcium silicate-based sealers demonstrated cell viability values above the ISO 10993-5 threshold of 70%, indicating low cytotoxicity. However, several sealers showed variable cytotoxic responses depending on experimental conditions.



Table 3. Cytotoxicity assessment results of calcium silicate-based sealer

No.	Sealer	Cell viability	ISO
1.	EndoSequence BC Sealer ^{13,17,29,30}	80-105%	
2.	EndoSequence BC Sealer HiFlow ^{23,28}	90-105%	
3.	GuttaFlow ²³	101-103%	
4.	BioRoot RCS ^{13,18,19}	73-120%	
5.	VDW.1Seal ²³	77-80%	
6.	One-Fil ³¹	83-121%	
7.	AH Plus Bioceramic Sealer ^{14,17,19,29,30}	82-110%	
8.	Bio-C Sealer ^{15,26,30}	77-100%	
9.	Bio-C Sealer ION+ ²⁸	94-100%	>70%
10.	Sealer Plus BC ²⁵	105-144%	
11.	TotalFill BC RRM Putty ²⁷	100%	
12.	Bio MTA+ ²⁶	76-94%	
13.	ProRoot ES ¹⁸	102%	
14.	BrightEndo MTA ³¹	75-100%	
15.	iRoot SP ²⁰	75-105%	
16.	EndoSeal TCS ³¹	100-114%	
17.	CeraSeal ^{21,31}	100-107%	
18.	BioRoot Flow ^{14,19}	30-103%	
19.	EndoSeal MTA ^{13,21,22}	28-104%	
20.	TotalFill BC Sealer ^{15,19,24,27,30}	50-100%	
21.	Well-Root ST ¹⁶	66%	<70%
22.	MTA Fillapex ^{18,20,22,25}	34-50%	
23.	NeoSEALER ²⁴	51-71%	

Table 4 summarizes the cytotoxicity assessment results of epoxy resin-based sealers, including cell viability values and their corresponding ISO classifications. These results illustrate the variability in cytotoxic responses observed among epoxy resin-based sealers across the included studies. As seen in Table 4, the cytotoxicity assessment of epoxy resin-based sealers demonstrated predominantly low cell viability values. Except for ADseal, which showed cell viability values above the ISO 10993-5 threshold, most epoxy resin-based sealers exhibited cell viability below 70%, indicating cytotoxic effects.

Table 4. Cytotoxicity assessment results of epoxy resin-based sealer

No.	Sealer	Cell viability	ISO
1.	ADseal ²³	77-85%	>70%
2.	AH Plus ^{13-20,22,24-31}	3-66%	
3.	AH Plus Jet ²⁹	15-21%	<70%
4.	AH26 ²¹	20-55%	



Discussions

The ideal root canal filling material should only reach the tooth apex without extending into the periradicular tissue. The application and flow of sealer to the periradicular tissue is difficult to control in some cases so that apical extrusion can occur.^{32,33} The sealer must have biocompatible properties because it is close to the periapical tissue which can affect the healing of the periradicular tissue.⁴ Cytotoxic sealers can cause a low healing rate when extrusion occurs.³³

Cytotoxicity is a disadvantage of epoxy resin-based sealers.¹⁶ Epoxy resin-based sealers are the gold standard for root canal obturation due to their physical properties and ability to seal root canals well.^{26,31} The cytotoxic properties of epoxy resin sealers can delay periapical healing of teeth with apical periodontitis.¹⁶ An alternative biocompatible sealer that is currently widely used is calcium silicate-based sealer.⁷⁻⁹ Calcium silicate-based sealers have many formulations that have been marketed commercially so that ongoing evaluation of the clinical safety of sealers is needed.¹⁰

Calcium silicate-based sealer has been shown to have a lower cytotoxic effect than epoxy resin-based sealer in the results of this systematic review study. The cytotoxic effect can be seen through the results of the sealer viability value.³⁴ The viability value of epoxy resin sealer is lower than calcium silicate sealer regardless of the cytotoxicity assessment method used in the study.¹³⁻³¹

The ISO 10993-5 standard states that a biomaterial is considered cytotoxic if the viability value is <70% and is considered non-cytotoxic if the viability value is >70%.³⁴ Several viability values of calcium silicate sealer brands are still below the ISO standard and one brand of epoxy resin sealer is above the ISO standard. Viability values can be influenced by several factors; the components of the sealer, the condition of the material when tested, and the exposure time in the study.¹⁹

The first factor that can affect the viability value is the sealer component. Research conducted by Lopez-Garcia et al. (2019), Omaia et al. (2023), Noites et al. (2023), and Sheila et al. (2023) stated that the cytotoxicity of epoxy resin sealer is caused by the epoxy resin and amine components. The addition of amine aims to accelerate the polymerization of epoxy resin. The reaction of epoxy resin with amine produces a toxic by-product, namely formaldehyde.^{15,16,26,27} AH Plus is one of the brands of epoxy resin sealer that is claimed to no longer release formaldehyde. This statement differs from the results of research by Sheila et al



(2023) which states that AH Plus still releases formaldehyde in very minimal concentrations.²⁷ This statement explains the viability value of AH Plus which is below the ISO standard as showed in Table 4.

The only epoxy resin sealer brand that meets ISO standards in this study is ADSeal. Research by Saber et al. (2023) obtained ADSeal viability values that meet ISO standards. The biocompatibility of ADSeal is due to the presence of calcium phosphate components that can induce bone formation.²³ This is in line with the results of research conducted by Kim et al. (2022) which stated that ADSeal is more biocompatible than AH Plus in fresh mixed conditions and after setting.³⁵

The sealer component is the cause of biocompatibility in calcium silicate sealers. Research by S6 et al. (2022) and Sebastian et al. (2024) explained that good biocompatibility in calcium silicate sealers is caused by bioceramic components, calcium hydroxide, and propylene glycol. This component can support the biological activity of periodontal ligament cells and promote periapical tissue repair.^{24,25} The sealer component is also the cause of the persistence of cytotoxic effects in calcium silicate sealers.

Additional components in calcium silicate sealer brands can cause increased cytotoxic effects on cells. The calcium silicate sealer brand that shows cytotoxic effects and has a viability value below the ISO standard is MTA Fillapex.^{18,20,22,25} MTA-based sealers are ideally biocompatible and can promote mineralization. The toxic component in MTA Fillapex is salicylate resin. This resin component is the cause of increased cell death.²⁰ Other cytotoxic components that affect the sealer viability value are found in EndoSeal MTA and BioRoot Flow. EndoSeal MTA has radiopacifier components in the form of toxic aluminum and bismuth.^{13,21} BioRoot Flow with a thickener component in the form of toxic acrylamide.¹⁹ Research by Celik et al. (2018) states that acrylamide can cause disruption of the oxidative stress balance in cells to cell death.³⁶

The second factor that can affect the viability value is the condition of the material (fresh mixed or after setting) when tested.¹⁹ Fresh mixed sealer testing on EndoSeal MTA, Well-Root ST, TotalFill BC Sealer and NeoSEALER brands showed lower viability values compared to sealer testing after setting.^{13,16,21,24,26} The fresh mixed condition of calcium silicate sealer causes the release of calcium hydroxide which results in high alkalinity. Uncontrolled high alkalinity can cause an inflammatory response that results in denaturation and damage to the surrounding cell membrane.³⁷ Although it has a negative impact, high alkalinity has the



potential for biological benefits. This is because the release of calcium ions can have a positive effect on encouraging periapical tissue repair and inhibiting osteoclast activity.^{16,24} High alkalinity that is not excessive can activate alkaline phosphatase for the tissue mineralization process.³⁷

The third factor that affects the viability value is the exposure time of the sealer. The exposure time factor is related to the components and conditions of the sealer. Viability values decrease with exposure time found in calcium silicate and epoxy resin sealers.²⁴ Fresh mixed calcium silicate sealer showed a decrease in viability value over time due to high alkalinity. Fresh mixed epoxy resin sealer showed a decrease in viability value over time due to toxic byproducts such as formaldehyde.²⁷

All reviewed journals concluded that the cytotoxic effect of calcium silicate-based sealers was lower than that of epoxy resin-based sealers, except for MTA Fillapex.¹³⁻³¹ MTA Fillapex had viability values similar to epoxy resin-based sealer brands.^{18,20,22,24} This systematic review concluded that calcium silicate-based sealers showed lower cytotoxicity than epoxy resin-based sealers, with the explanation that the components, conditions, and exposure time of the material play an important role in the biocompatibility of the sealer. Calcium silicate sealers can be a sealer of choice in root canal treatment cases that have the potential for extrusion, such as large apical widths, root resorption, or immature permanent teeth.^{20,31}

Conclusion

Calcium silicate-based sealer shows lower cytotoxicity compared to epoxy resin-based sealer. The results of this systematic review study do not directly conclude that calcium silicate sealer is an ideal sealer. The ideal requirement for a sealer is not only good biocompatibility. Further systematic review studies are needed to determine the comparison of the physical properties of calcium silicate-based sealers with epoxy resin-based sealers.

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Table 5. List of articles

No. Articles	Researcher	Analysis of bias	Year of publication	Cell lineage	Control groups	Sealers evaluated	Cytotoxicity assessment	Times of evaluation	Outcomes	
1.	Biocompatibility and Mineralization Activity of Three Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Dental Pulp Stem Cells	Seo et al. ¹³	<i>Low risk</i>	2019	Human dental pulp stem cells (hDPSCs)	hDPSCs + medium	AH Plus, EndoSequence BC Sealer, BioRoot RCS, Endoseal MTA	MTT assay	0h, 24h, 48h, 72h, 120h	At all evaluation time points, it was found that calcium silicate-based sealers, EndoSequence BC Sealer, BioRoot RCS, and Endoseal MTA showed the same cell viability as the control group. AH Plus showed low cell viability especially after 72h.
2.	Comparative bioactivity and immunomodulatory potential of the new Bioroot Flow and AH Plus Bioceramic sealer: An in vitro study on hPDLSCs	Sanz et al. ¹⁴	<i>Low risk</i>	2024	Human periodontal ligament stem cells (hPDLSCs)	hPDLSCs + medium (negative control) OsteoDiff media (positive control)	AH Plus, Bioroot Flow dan AH Plus Bioceramic Sealer	MTT assay	24h, 48h, 72h	AH Plus showed lower metabolic activity compared to the control group, while AH Plus Bioceramic Sealer and Bioroot Flow showed results that were not significantly different from the control group at all evaluation times.
3.	Comparative Cytocompatibility and Mineralization Potential of Bio-C Sealer and TotalFill BC Sealer	López-García et al. ¹⁵	<i>Low risk</i>	2019	Human periodontal ligament stem cells (hPDLSCs)	hPDLSCs + medium (negative control) OsteoDiff media (positive control)	Bio-C Sealer, TotalFill BC Sealer, AH Plus	MTT assay	24h, 48h, 72h	TotalFill BC Sealer and Bio-C Sealer showed significantly lower cytotoxic values than AH Plus at all evaluation times.
4.	Comparative Study of The	Omaia M dan	<i>Low risk</i>	2023	Human fibroblast	Human fibroblast +	AH Plus Sealer, Well-	MTT assay	24h	Calcium silicate-based sealer (Well-Root ST)



					medium (negative control)	Root ST Sealer				showed better cytocompatibility than epoxy resin-based sealer (AH Plus).
5.	Cytotoxic Effect of Epoxy Resin and Calcium Silicate Based Sealers (An in-vitro study)	Shaker M. ¹⁶								
5.	Cytocompatibility and bioactive potential of AH Plus Bioceramic Sealer: An in vitro study	Sanz et al. ¹⁷	<i>Low risk</i>	2022	Human periodontal ligament stem cells (hPDLSCs)	hPDLSCs + medium (negative control) OsteoDiff media (positive control)	AH Plus Bioceramic Sealer, AH Plus, Endosequence BC Sealer	MTT assay	24h, 48h, 72h	AH Plus showed significantly lower viability values compared to the control group at all evaluation times. AH Plus Bioceramic and Endosequence BC Sealer had a quite similar cell viability values to the control group.
6.	Cytokine Production and Cytotoxicity of Calcium Silicate based Sealers in 2- and 3-dimensional Cell Culture Models	Gaudin et al. ¹⁸	<i>Low risk</i>	2020	Human periodontal ligament stem cells (hPDLSCs)	hPDLSCs + medium (negative control) Eagle medium dengan 20% dimethyl sulfoxide (positive control)	BioRoot RCS, ProRoot ES, AH Plus, MTA Fillapex,	MTT assay	24h	BioRoot RCS and ProRoot ES showed better cytocompatibility compared to MTA Fillapex and AH Plus.
7.	Cytotoxicity and genotoxicity of bioceramic root canal sealers compared to conventional resin-based sealer	Radwanski et al. ¹⁹	<i>Low risk</i>	2024	Human gingival fibroblasts (hGF) Macrophage peripheral	Sel + medium (negative control) 100% dimethyl	AH Plus, Total Fill BC Sealer, AH Plus Bioceramic Sealer, BioRoot Flow, BioRoot RCS	XTT assay	24h, 48h	AH Plus showed 90% viability value on hGF and SC cells in both incubation periods. BioRoot Flow showed moderate local cytotoxicity as it only significantly reduced the viability of hGF cells



blood cell
line (SC) sulfoxide
(DMSO)

compared to the negative control. BioRoot RCS showed higher cytotoxicity value than AH Plus on hGF (local cytotoxicity). AH Plus and AH Plus Bioceramic Sealer did not show any cytotoxicity (>90%) on the tested cells.

iRootSP shows higher viability values than AHPlus and MTA-Fillapex.

8.	Cytotoxicity and genotoxicity of salicylate- and calcium silicate-based root canal sealers on primer human periodontal ligament fibroblasts	Erdogan et al. ²⁰	<i>Moderate risk</i>	2021	Primary human periodontal ligament fibroblast cell (hPDLFC)	hPDLFC + medium	AH Plus, MTA Fillapex, iRootSP	XTT assay	0h, 6h, 12h, 24h, 48h, 72h	iRootSP shows higher viability values than AHPlus and MTA-Fillapex.
9.	Cytotoxicity and Mineralization Activity of Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Gingival Fibroblast Cells	Shokrzadeh et al. ²¹	<i>Moderate risk</i>	2023	Human gingival fibroblast cells (HGF)	-	AH26, Ceraseal, Endoseal MTA	MTT assay	24h, 48h, 72h, 120h	Endoseal MTA and Ceraseal showed lower cytotoxicity values than AH26. There was no significant difference between the cytotoxicity values of Endoseal MTA and Ceraseal.
10.	Cytotoxicity of Two Calcium Silicate-Based Sealers and AH Plus Resin-Based Sealer for Human	Hoshyari et al. ²²	<i>Low risk</i>	2022	Human gingival fibroblasts (HGFs)	HGFs + medium	AH Plus, Endoseal MTA, MTA Fillapex	MTT assay	4h	Endoseal MTA showed lower cytotoxicity compared to MTA Fillapex and AH Plus



Study No.	Study Title	Author	Risk	Year	Cell Type	Medium	Sealer	Assay	Time	Conclusion
11.	Gingival Fibroblasts Effect of Different Sealers on the Cytocompatibility and Osteogenic Potential of Human Periodontal Ligament Stem Cells: An In Vitro Study	Saber et al. ²³	Low risk	2023	Human periodontal ligament stem cells (hPDLSCs)	hPDLSCs + medium	VDW.1Seal, Endosequence BC Sealer, HiFlow, GuttaFlow, ADSeal	MTT assay	24h, 48h, 72h	GuttaFlow and VDW.1Seal showed the highest percentage of cell viability.
12.	Evaluation of the antibacterial and cytotoxic properties of TotalFill and NeoSEALER flo bioceramic sealers	Sebastian et al. ²⁴	Low risk	2024	Human gingival fibroblasts	Sel + DMEM media (negative control) DMEM (positive control)	TotalFill BC Sealer, NeoSEALER Flo, AH Plus	MTT assay	24h, 48h, 120h	TotalFill and NeoSEALER Flo showed better biocompatibility compared to AH Plus. NeoSEALER showed significantly higher cell viability compared to AH Plus
13.	Genotoxicity and Cytotoxicity Comparison of Calcium Silicate-Based and Resin-Based Sealers on Human Periodontal Ligament Stem Cells	Só et al. ²⁵	Low risk	2022	Human periodontal ligament dental stem cells (hPDLSCs)	hPDLSCs + medium	Sealer Plus BC, AH Plus, MTA Fillapex	MTT assay	24h, 48h, 72h	Sealer Plus BC showed the lowest cytotoxicity value followed by the control group, MTA Fillapex and AH Plus.
14.	Human Gingival Fibroblasts Response to Different Endodontic	Noites et al. ²⁶	Low risk	2023	Human gingival fibroblast (HGF)	HGF + medium	AH Plus, Bio MTA+, Bio C Sealer	MTT assay	24h, 72h	After 24h, the cytotoxic effect was only shown by AH Plus and increased at 72h. The cytotoxic effect was not seen in Bio MTA+



15.	Sealers: An In Vitro Study In Vitro Cytotoxicity and Mineralization Potential of an Endodontic Bioceramic Material	Sheela et al. ²⁷	<i>Low risk</i>	2023	Primary human osteoblast (HOB)	HOB + medium	AH Plus, TotalFill BC RRM putty, TotalFill BC Sealer	XTT assay	24h	and Bio C sealer after 24h, but slightly cytotoxic at 72h. At low concentrations, TotalFill BC showed biocompatibility on HOB cells compared to AH Plus.
16.	Microstructural composition, ion release, and bioactive potential of new premixed calcium silicate-based endodontic sealers indicated for warm vertical compaction technique	Sanz et al. ²⁸	<i>Low risk</i>	2020	Human periodontal ligament cells (hPDLs)	hPDLs + medium	Bio-Csealer ION+, EndoSequence BC Sealer HiFlow, AH Plus	MTT assay	24h, 48h, 72h	BCHiF showed good biocompatibility with higher values compared to control. AH Plus showed cytotoxic effects at each test time period.
17.	Physicochemical and Biological Properties of AH Plus Bioceramic	Souza et al. ²⁹	<i>Low risk</i>	2023	Human periodontal ligament (HPDL) fibroblast	Sel + medium (negative control) 0,1% sodium dodecyl sulfate (positive control)	AH Plus Bioceramic, AH Plus Jet, EndoSequence BC Sealer	XTT assay	24h	AH Plus showed lower cell viability than the value set by the ISO standard. AH Plus Bioceramic and Endosequence BC Sealer showed higher values than the ISO standard, which was more than 70%.
18.	Physicochemical Properties and Biocompatibility of Various Bioceramic Root	Kwak et al. ³⁰	<i>Moderate risk</i>	2023	Human periodontal ligament fibroblast (hPDLF)	AH Plus	AH Plus Bioceramic Sealer, EndoSequence BC Sealer, TotalFill BC	MTT assay	1d, 2d, 3d, 7d	Calcium silicate-based bioceramic sealers (AH Plus Bioceramic sealer, Endosequence BC, TotalFill BC, Bio-C Sealer) show



Canal Sealers: In Vitro Study						Sealer, Bio-C Sealer, AH Plus			better biocompatibility compared to AH Plus
19. Physicochemical properties and cytocompatibility of newly developed calcium silicate-based sealers	Park et al. ³¹	<i>Low risk</i>	2021	Human periodontal ligament fibroblast (hPDLF)	AH Plus Jet	BrightEndo MTA sealer, CeraSeal, EndoSeal TCS, One-Fil, AH Plus	MTT assay	1d, 2d, 3d, 7d	The cell viability value of calcium silicate-based sealers (BrightEndo MTA sealer, CeraSeal, EndoSeal TCS, One-Fil) was higher than AH Plus in fresh extraction. While in extraction after setting, both showed viability values that tended to be the same.



Table 6. Bias analysis results

No	Studies	Description of cell lineage	Control group	Description of cell culture preparation	Description of sealer preparation	Reproducibility of the study	Description of statistical analysis	Result
1.	Seo et al. ¹³	YES	YES	YES	YES	YES	YES	LOW
2.	Sanz et al. ¹⁴	YES	YES	YES	YES	YES	YES	LOW
3.	Lopez-Garcia et al. ¹⁵	YES	YES	YES	YES	YES	YES	LOW
4.	Omaia et al. ¹⁶	YES	YES	YES	YES	YES	YES	LOW
5.	Sanz et al. ¹⁷	YES	YES	YES	YES	YES	YES	LOW
6.	Gaudin et al. ¹⁸	YES	YES	YES	YES	YES	YES	LOW
7.	Radwanski et al. ¹⁹	YES	YES	YES	YES	YES	YES	LOW
8.	Erdogan et al. ²⁰	YES	YES	YES	NO	NO	YES	MODERATE
9.	Shokrzadeh et al. ²¹	YES	NO	YES	YES	NO	YES	MODERATE
10.	Hoshyari et al. ²²	YES	YES	YES	YES	NO	YES	LOW
11.	Saber et al. ²³	YES	YES	YES	YES	YES	YES	LOW
12.	Sebastian et al. ²⁴	YES	YES	YES	YES	YES	YES	LOW
13.	So et al. ²⁵	YES	YES	YES	YES	YES	YES	LOW
14.	Noites et al. ²⁶	YES	YES	YES	YES	NO	YES	LOW
15.	Sheela et al. ²⁷	YES	YES	YES	YES	YES	YES	LOW
16.	Sanz et al. ²⁸	YES	YES	YES	YES	YES	YES	LOW
17.	Souza et al. ²⁹	YES	YES	YES	YES	YES	YES	LOW
18.	Kwak et al. ³⁰	YES	YES	NO	YES	NO	YES	MODERATE
19.	Park et al. ³¹	YES	YES	YES	YES	NO	YES	LOW