ARE CALCIUM SILICATE SEALERS LESS CYTOTOXIC AND GENOTOXIC THAN EPOXY RESIN SEALERS? A SYSTEMATIC REVIEW OF IN VITRO STUDIES.

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ABSTRACT

Aims: This systematic review aimed to evaluate whether calcium silicate-based sealers are less cytotoxicity and genotoxicity than epoxy resin-based sealers.

Materials and Methods: Systematic searches were conducted for studies published up to September 27th, 2022, without restriction for language or year of publication, in the following databases: MEDLINE/PubMed, Scopus, Web of Science and Grey Literature Report. Only *in vitro* studies that evaluated the cytotoxicity or genotoxicity of calcium silicate and epoxy resin-based sealers were included. The quality assessment was performed.

Results: After duplicate removal and eligibility criteria assessment, a total of thirty-four studies were included. Twenty-eight studies had a low risk of bias, and six studies had a moderate risk of bias. In general, calcium silicate-based sealers had a lower cytotoxic and genotoxic potential than epoxy-resin based sealers.

Conclusions: Based on the findings from *in vitro* studies, calcium silicate-based sealers are less cytotoxic and genotoxic than epoxy resin-based sealers.

KEYWORDS: Calcium silicate. Cytotoxicity. Epoxy resin. Genotoxicity. Systematic review.

INTRODUCTION

After root canal preparation, filling the space previously occupied by the dental pulp is mandatory[1]. If not correctly filled, empty spaces can serve to develop resistant microorganisms, establish a persistent infection, or access the root canal and periapical tissues, establishing a secondary infection[1,2]. Thus, the proper sealing of the root canal system is necessary for the long-term success of the endodontic treatment[3].

For this purpose, endodontic sealers are used in root canal filling and must present properties that allow them to promote good sealing, such as dimensional stability, low solubility, and adequate flow[2,4]. In addition, these sealers must present biocompatibility[5–7]. A biocompatible material usually is defined as showing a low or no cytotoxicity, not promoting adverse reactions when in contact with periapical tissues[4]. Besides, it must possess low genotoxicity, which is defined as the capacity of the material to act on the cellular genetic code, being, therefore, an essential indicator of carcinogenic potential[8,9].

Mainly for possessing good physicochemical properties, epoxy resin-based sealers are considered the gold standard sealers in endodontics[10]. However, still presenting some level of

cytotoxicity[1] and genotoxicity[6]. Calcium silicate-based sealers appear like an alternative, presenting physicochemical properties comparable to epoxy resin sealers[11,12], under the premise of presenting better biological properties.

Different formulations of calcium silicate-based sealers have been released to the market over the past years, constantly evaluating their properties to determine their clinical safety. Therefore, this systematic review aimed to answer the following question: "Are the cytotoxicity and genotoxicity of calcium silicate-based sealers comparable to epoxy resin-based sealers?"

MATERIALS AND METHODS

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) recommendations[13]. Since this is a systematic review of *in vitro* studies, PROSPERO registration is not available.

Search strategy

Two independent authors (V.S.E. and T.W.) conducted the searches on the following electronic databases: MEDLINE/PubMed, Scopus, Web of Science, and Grey Literature Report. Studies published from inception up to September 27th, 2022, without restriction for language or year of publication, were selected. The electronic searches were developed using the most cited descriptors in previous publications on this theme combining Medical Subject Heading (MeSH) terms and text words (tw.). For each database, the following terms were combined, according to the investigated outcome: "calcium silicate", "calcium silicate sealer", "bioceramic", "epoxy resin", "epoxy resin sealer"; "cytotoxicity", "cytocompatibility", "biocompatibility", "cell viability", "cell proliferation", "genotoxicity", "micronuclei", "foci" and "DNA damage". The Boolean operators' AND' and 'OR' were used to combine the terms. Searches performed in each database are summarized in **Table 1** (cytotoxicity) and **Table 2** (genotoxicity). All studies were imported into the Mendeley© (Mendeley Ltd, London, United Kingdom) reference manager to catalog and facilitate the exclusion of duplicates.

Table 1. Search strategy in each database for studies investigating cytotoxicity.

Database	Search strategy	Findings
MEDLINE/PubMed	#1: ((Calcium Silicate) OR (Calcium Silicate Sealer)) OR (Bioceramic)	4.925
	#2: (Epoxy Resin) OR (Epoxy Resin Sealer) OR (AH Plus)	9.061
	#3: ((((Cytotoxicity) OR (Cytocompatibility)) OR (Biocompatibility)) OR (Cell Viability)) OR (Cell Proliferation)	1.647.014
	#1 AND #2 AND #3	56
Scopus	#1: (ALL (calcium AND silicate) OR ALL (calcium AND silicate AND sealer) OR ALL (bioceramic))	142.881
	#2: (ALL (epoxy AND resin) OR ALL (epoxy AND resin AND sealer) OR ALL (ah AND plus))	243.057
	#3: (ALL (cytotoxicity) OR ALL (cytocompatibility) OR ALL (biocompatibility) OR ALL (cell AND viability) OR ALL (cell AND proliferation))	3.506.864
	#1 AND #2 AND #3	1.522
Web of Science	#1: TS=(Calcium Silicate OR Calcium Silicate Sealer OR Bioceramic)	18.395
	#2: TS=(Epoxy Resin OR Epoxy Resin Sealer OR AH Plus)	50.463
	#3: TS=(Cytotoxicity OR Cytocompatibility OR Biocompatibility OR Cell Viability OR Cell Proliferation)	1.037.668
	#1 AND #2 AND #3	66
Grey Literature Report	#1: Calcium Silicate OR Calcium Silicate Sealer OR Bioceramic	0
	#2: Epoxy Resin OR Epoxy Resin Sealer OR AH Plus	0
	#3: Cytotoxicity OR Cytocompatibility OR Biocompatibility OR Cell Viability OR Cell Proliferation	0
	#1 AND #2 AND #3	0

Table 2. Search strategy in each database for studies investigating genotoxicity.

Database	Search strategy	Findings
MEDLINE/PubMed	#1: ((Calcium Silicate) OR (Calcium Silicate Sealer)) OR (Bioceramic)	4.925
	#2: (Epoxy Resin) OR (Epoxy Resin Sealer) OR (AH Plus)	9.061
	#3: (((Genotoxicity) OR (Micronuclei)) OR (Foci)) OR (DNA damage)	255.155
	#1 AND #2 AND #3	3
Scopus	#1: (ALL (calcium AND silicate) OR ALL (calcium AND silicate AND sealer) OR ALL (bioceramic))	142.881
	#2: (ALL (epoxy AND resin) OR ALL (epoxy AND resin AND sealer) OR ALL (ah AND plus))	243.057
	#3: (ALL (genotoxicity) OR ALL (micronuclei) OR ALL (foci) OR ALL (dna AND damage))	4.844.337
	#1 AND #2 AND #3	658
Web of Science	#1: TS=(Calcium Silicate OR Calcium Silicate Sealer OR Bioceramic)	18.395
	#2: TS=(Epoxy Resin OR Epoxy Resin Sealer OR AH Plus)	50.463
	#3: TS=(Genotoxicity OR Micronuclei OR Foci OR DNA damage)	1.854.516
	#1 AND #2 AND #3	5
Grey Literature Report	#1: Calcium Silicate OR Calcium Silicate Sealer OR Bioceramic	0
	#2: Epoxy Resin OR Epoxy Resin Sealer OR AH Plus	0
	#3: Genotoxicity OR Micronuclei OR Foci OR DNA damage	0
	#1 AND #2 AND #3	0

Eligibility criteria

Eligibility criteria for study selection were based on the PICOS strategy (PRISMA-P 2015)[13–15], as follows:

- Population (P): cellular model;
- Intervention (I): samples of calcium silicate-based sealers;
- Comparison (C): samples of epoxy resin-based sealers;
- Outcome (O): cytotoxicity (e.g. cell viability/proliferation); genotoxicity (e.g. micronuclei formation, foci alteration and/or DNA damage);
- Study Design (S): *in vitro* studies.

Only *in vitro* studies that compared the cytotoxicity or genotoxicity of calcium silicate-based sealers to epoxy resin-based sealers were included.

Studies that compared other properties of the sealers evaluated, studies that evaluated experimental sealers, studies performed in animals, reviews with and without meta-analysis, case, and serial reports, longitudinal and cross-sectional studies, randomized and non-randomized studies were excluded.

Study selection

Two authors (V.S.E. and T.W.) were responsible for the study selection. The first stage consisted of duplicates exclusion and selecting studies by title and abstract reading. When it was impossible to determine the study inclusion by title and abstract reading, the full text was accessed and read for the final decision. The second stage consisted of the full-text reading of the potentially eligible studies based on the eligibility criteria adopted in the PICOS strategy. Finally, disagreements were solved by consensus with a third author (M.V.R.S.).

Data extraction

Once again, two authors (V.S.E. and T.W.) were responsible for extracting the data from the included studies. Disagreements were solved by consensus with a third author (M.V.R.S.). The following data were extracted: author(s), year of publication, sealers evaluated, control group, cell lineage, extracts dilutions, the method for cytotoxicity assessment, the method for genotoxicity assessment, times of evaluation, main findings. In case of need missing information, author(s) were contacted by e-mail at least three times to obtain additional information.

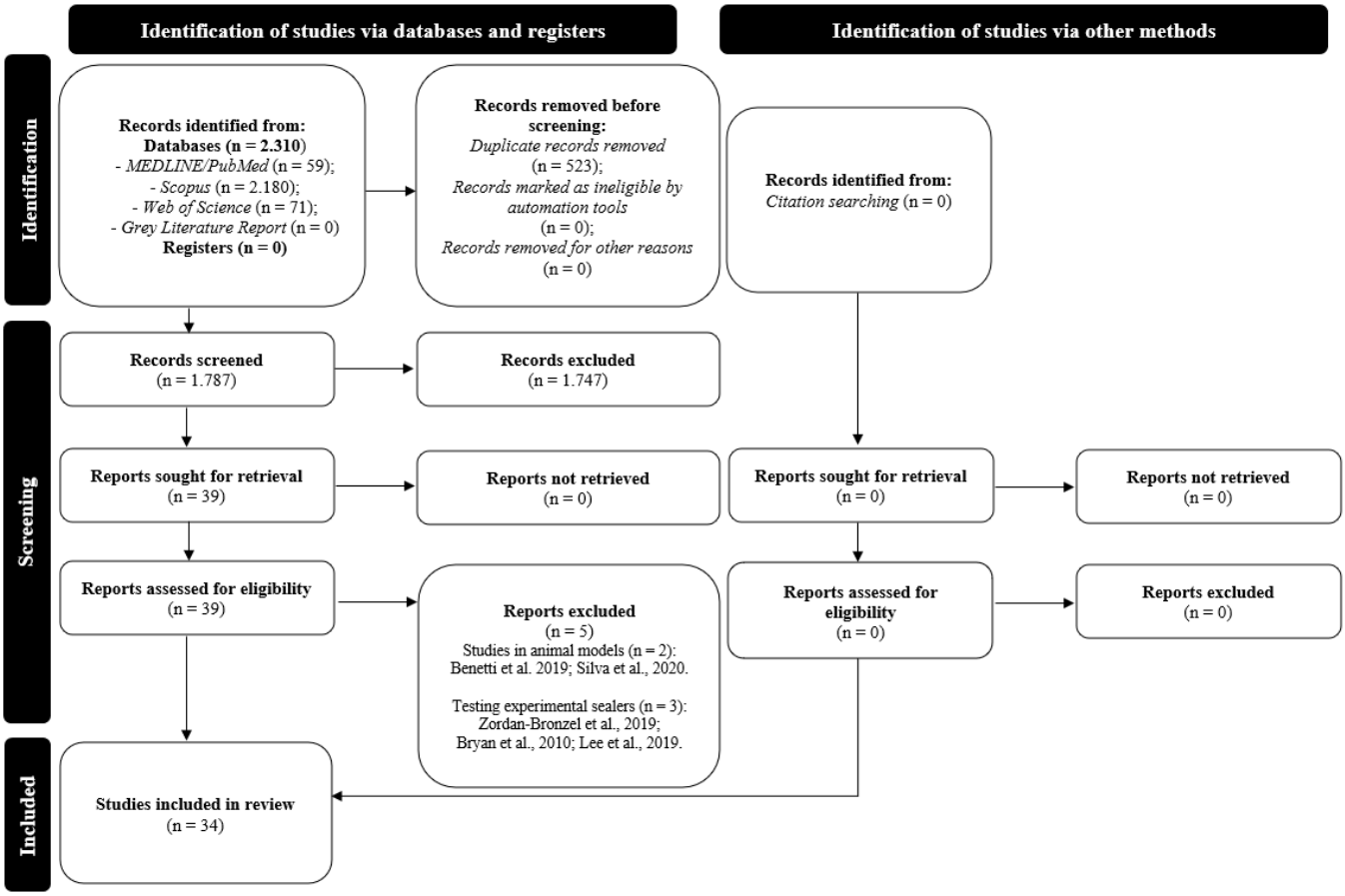

Figure 1. PRISMA flow diagram of the systematic searches.

Risk of bias assessment

The risk of bias of the included studies was assessed through an adaptation of two previous systematic reviews[16,17]. The following parameters were used to assess the risk of bias for each study: description of cell lineage, presence of a control group, description of the cell culture preparation, description of sealer/extracts preparation, reproducibility of experiments (e.g., tests performed in duplicate, triplicate), and description of statistical analysis.

It was attributed a 'yes' where the parameter was found and a "no" in the absence of the parameter. Articles where only one to two of these parameters were found were classified as presenting a "high" risk of bias; the presence of three to four parameters was considered as a "moderate" risk of bias; the presence of five to six parameters was considered as a "low" risk of bias. Two authors (V.S.E. and T.W.) independently evaluated the methodological quality of each included study. In case of disagreements, a third author (M.V.R.S.) validated the analysis.

RESULTS

Study selection

Initial screening of databases resulted in 2.310 studies, where 523 were excluded for duplicates, as presented in the flow diagram (**Figure 1**). After title and abstract reading, 39 studies were selected for full-text reading.

After full-text reading, five studies[11,18-21] were excluded. Two for being studies in animal models[11,18], and three for testing experimental sealers[19-21]. Therefore, thirty-four studies were included in the present systematic review[22–55].

Data extraction

Table 3 shows the characteristics and main findings of the studies included in this review.

Authors of studies with missing information were contacted by e-mail three times. However, no additional information was obtained.

The calcium silicate sealers evaluated were the TotalFill BC Sealer[24,28,30,33,42,52]; BioRoot RCS[23-26,30,31,35,38,46]; ProRoot ES[25,36]; ProRoot[41]; iRoot SP[22,35,37,48]; EndoSequence BC Sealer[23,27,29,32,34,36,40,43,45-47,49,50,53,54]; EndoSequence BC Sealer HiFlow[45,54]; Sealer Plus BC[51,52,55]; C-Root[38]; Well-Root ST[39]; Bio-C Sealer[42]; Bio-C Sealer ION+[29]; EndoSeal TCS[44]; CeraSeal[44]; and AH Plus Bioceramic[53].

As for the epoxy resin sealers evaluated, these were the AH Plus[22-34,36,38,39-48,50-55]; AH Plus JET[35,37,49];

Sealer Plus[51]; Acroseal[35]; and EasySeal[24].

Regarding cell lineages, studies performed their investigations using the following lineages: human bone marrow-derived mesenchymal stem cells (hMSCs)[23]; human gingival fibroblast cells (FMM1 cell lineage)[34]; human gingival fibroblast-1 (HGF-1)[24]; periodontal ligament cell line using lentiviral gene transfer of human telomerase reverse transcriptase (PDLhTERT)[35]; human periodontal ligament fibroblast cell (hPDLFC)[22,49,54]; apical papillary cells (APCs)[51]; human periodontal ligament stem cells (hPDLSCs)[25,28,38,39,42,44,45,53,55]; human tooth germ stem cells (hTGSCs)[37]; 3T3 fibroblasts[27,31,47]; human periodontal ligament cells (hPDLCs)[29,30]; human dental pulp stem cells (hDPSCs)[46]; human gingival fibroblasts[32]; human osteoblast-like cells (Saos-2)[52]; NIH3T3 murine fibroblasts[33]; murine osteoblast precursor (IDG-SW3) cell line[36]; murine calvaria pre-osteoblast cell line (MC3T3-E1) cells[40,41,42]; L929 mouse fibroblasts[50]; and lymphocytes[48].

As for the extracts' dilutions, sixteen studies did not report for the dilutions tested[24,30,33,34,36-38,41,43,44,46-50,54]. Eighteen studies used dilutions up to 1:50[22,23,25-29,31,32,35,39,40,42,45,51-53,55]; while three studies tested dilutions greater than 1:50[32,35,40].

The following cytotoxicity tests were performed: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[25-29,33,34,41-43,45-47,50-53,55]; Alamar blue assay[23,49]; formazan dye / ELISA[24]; XTT-based cell viability assay[22,35,54]; sulforhodamine B (SRB) assay[51]; modified staining sulforhodamine B assay[31]; a luminescence assay based on adenosine triphosphate quantification[36]; 3-(4, 5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium (MTS) assay[37]; cell counting kit-8 (CCK-8) assay[38,39,44]; a living-cell-count, living/dead staining, and LDH-assay[26]; EZ-Cytox-enhanced cell viability assay[40]; DNA-specific fluorochrome Hoechst 33342[28]; Costar Transwell cell viability assay[30]; quantitative flow cytometry test[32]; neutral red assay[52].

The following genotoxicity tests were performed: micronucleus formation (MN) test[22,34,55]; γ-H2AX assay[35,48].

In general, the majority of the studies reported a lower cytotoxic effect for the investigated calcium silicate-based sealers[22-42,44-46,49-55]. Only two studies did not report differences among the investigated sealers[43,47].

Also, the majority of studies that investigated sealers genotoxicity reported a lower genotoxic effect for the investigated calcium-silicate based sealers[22,34,35,48]. Only one study did not report differences among the investigated sealers[55].

Risk of bias assessment

Table 4 presents the risk of bias of each included study. According to the parameters evaluated, the majority of studies had a low risk of bias. Only six studies had a moderate risk of bias[24,37,38,41,47,48], for not reporting for the following parameters: "presence of a control group"[41], "description of sealers/extract preparation"[24,37,38,41,47,48], "reproducibility of experiments"[24,37,38,41,47,48], and "description of statistical analysis"[24].

DISCUSSION

Sealers used for the root canal filling usually are in close contact with the periapical tissues, requiring them to be biologically viable[5–7]. So far, epoxy resin-based sealers are considered to be the gold standard sealers in endodontics[10]. Mainly due to this reason, in the present systematic review, these sealers were considered as the comparison group. However, epoxy resin-based sealers still present some degree of cytotoxicity and genotoxicity[1,6]. Due to this reason, with the premise of presenting a lower toxic potential, calcium silicate-based sealers emerged as a more biologically compatible alternative[11]. Although previous systematic reviews on this subject exists[10,56], the constant release of new materials and the consequent publication of new studies that evaluate such materials make this systematic review necessary.

Through the results presented by the included studies, it is possible to determine that regardless of the method used to access cytotoxicity, calcium silicate-based sealers are less cytotoxic than epoxy resin-based sealers[22-42,44-46,49-55]. This higher cytotoxicity presented by the epoxy resin sealers is probably associated with the initial release of formaldehyde from the amines added to accelerate the epoxy resin polymerization process, which is a mutagenic substance[12]. Only two studies did not report differences among the investigated sealers cytotoxicity results[43,47]. However, these results may be related to the methodologies adopted. In one study, the authors evaluated sealers after setting during a 6-week period using a murine cell lineage[43].

Table 3. Characteristics of the included studies.

Author(s)/ Year of publication	Sealers evaluated	Cell lineage	Control groups	Extract dilution	Cytotoxicity assessment	Genotoxicity assessment	Times of evaluation	Outcomes	Main Findings
Almeida et al., 2020 (33)	TotalFill BC Sealer; AH Plus; MTA Fillapex	NIH3T3 murine fibroblasts	Dulbecco's Modified Eagle's Medium (DMEM) + 10% Fetal Bovine Serum (FBS);	NR	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay	-	24, 48, 72hs	AH Plus and MTA Fillapex were more cytotoxic than TotalFill BC Sealer	TotalFill BC Sealer had the lowest cytotoxicity among the investigated sealers
Alsubait et al., 2018 (23)	BioRoot RCS; EndoSequence BC Sealer; AH Plus	Human bone marrow-derived mesenchymal stem cells (hMSCs)	hMSCs + medium	1:2; 1:8; 1:32	Alamar blue assay	-	1, 3, 7 days	AH Plus cell viability was lower than the control at all periods; No differences were found between calcium silicate sealers and control; AH Plus cell viability was lower than the calcium silicate sealers, except in 1:32 at day 1, and 1:2 at day 7	Calcium silicate sealers were less cytotoxic than AH Plus
Candeiro et al., 2016 (34)	EndoSequence BC Sealer; AH Plus BioRoot RCS;	Human gingival fibroblast cells (FMM1 cell lineage)	FMM1 cells + medium	NR	MTT assay	Micronucleus formation (MN) test	1, 24, 72, 168hs	CYTOTOXICITY EndoSequence BC Sealer had significantly higher cell viability than AH Plus; GENOTOXICITY EndoSequence BC Sealer had a significantly smaller percentage of cells with micronucleus than AH Plus	EndoSequence BC Sealer had lower cytotoxicity and genotoxicity compared with AH Plus
Colombo et al., 2018 (24)	TotalFill BC Sealer; AH Plus; EasySeal; MTA Fillapex; Sealapex	Human gingival fibroblast-1 (HGF-1)	HGF-1 + medium	NR	Formazan dye / ELISA	-	24, 48, 72hs	EasySeal and MTA Fillapex showed severe cytotoxic activity, AH Plus and Sealapex moderate cytotoxicity, and BioRoot RCS and TotalFill BC Sealer were both cytocompatible	Bioceramic sealers have greater cytocompatibility compared to the other tested sealers

Eldeniz et al., 2016 (35)	BioRoot RCS; iRootSP; AH Plus JET; Acroseal; EndoREZ; RealSeal; RealSeal SE; Hybrid Root SEAL; MTA Fillapex	Periodontal ligament cell line using lentiviral gene transfer of human telomerase reverse transcriptase (PDLhTERT)	CYTOTOXICITY PDLhTERT + medium (negative control); PDLhTERT + medium + 1% Triton X-100 (positive control); GENOTOXICITY PDLhTERT + medium (negative control); PDLhTERT + medium + 1 mmol L^{-1} H_2O_2 (positive control)	CYTOTOXICITY 1:3; 1:10; 1:30; 1:100; 1:300 GENOTOXICITY 1:3 1:10	XTT-based cell viability assay	γ-H2AX assay	24hs	CYTOTOXICITY Undiluted sealers were cytotoxic, except AH Plus JET, RealSeal SE, iRootSP and BioRoot RCS; BioRoot RCS and iRootSP were the least cytotoxic sealers; GENOTOXICITY Hybrid Root SEAL induced greater γ-H2AX formation compared to negative control; Both concentrations of AH Plus JET, Acroseal, RealSeal and MTA Fillapex sealers had no difference compared to the negative control; Highest rates of DSBs (foci) were found in BioRoot RCS and RealSeal SE when PDLhTERT cells were exposed to 1/3; 1/10 Bio-Root RCS and RealSeal SE had less DSBs formation compared to negative control; and iRootSP had more DSBs at the same concentration	Bioceramic sealers were the less toxic sealers tested
Erdogan et al., 2021 (22)	iRoot SP; AH Plus; MTA Fillapex	Human periodontal ligament fibroblast cell (hPDLFC)	hPDLFC culture + medium	1:1; 1:2; 1:4; 1:8; 1:16; 1:32	XTT-based cell viability assay	MN test	0, 6, 12, 24, 48, 72hs	CYTOTOXICITY AH Plus and MTA Fillapex had the lowest percentage of cell viability at 1:1, 1:2, 1:4; iRootSP showed higher viability at all concentrations and times than AHPlus and MTA Fillapex; GENOTOXICITY AHPlus increased the number of micronuclei; MTA Fillapex slightly induced micronucleus formation and iRoot SP was not increased	iRoot SP had lowest cytotoxic and genotoxic potential among the investigated sealers

Ferreira et al., 2022 (51)	Sealer Plus BC; AH Plus; Sealer Plus; MTA Fillapex	Apical papillary cells (APCs)	DMEM	1:10	MTT assay; Sulforhodamine B (SRB) assay	-	MTT: 24, 72hs; SRB: 72hs	MTT ASSAY: At 24hs, all sealers had similar cytotoxicity; After 72hs, Sealer Plus BC and Sealer Plus had higher cell viability; SRB ASSAY: Sealer Plus BC had the highest cell viability	Calcium-silicate sealers had higher cell viability among the investigated sealers
Gaudin et al., 2020 (25)	BioRoot RCS; ProRoot ES; AH Plus; MTA Fillapex	Human periodontal ligament stem cells (hPDLSCs)	Eagle medium with 20% dimethyl sulfoxide (positive control) hPDLSCs + medium (negative control)	1:1; 1:2; 1:4; 1:8	MTT assay	-	24hs	BioRoot RCS increased cell viability; ProRoot ES had no effect on cell viability; MTA Fillapex was strongly cytotoxic; AH Plus had a mild toxicity at a high concentration (1:1)	BioRoot RCS and ProRoot ES showed better cytocompatibility compared with MTA Fillapex and AH Plus
Giacomino et al., 2019 (36)	EndoSequence BC Sealer; ProRoot ES; AH Plus; Roth Sealer	Murine osteoblast precursor cell line (IDG-SW3)	IDG-SW3 + medium; Wells without cells	NR	Luminescence assay based on adenosine triphosphate quantification	-	7 days	Bioceramic sealers had greater cytocompatibility even at high concentrations; Cell death was detected when Roth and AH Plus were used at concentrations lower than the bioceramic sealers	EndoSequence BC Sealer and ProRoot ES were significantly more biocompatible
Güven et al., 2013 (37)	iRoot SP; AH Plus JET; MTA Fillapex	Human tooth germ stem cells (hTGSCs)	hTGSCs + medium	NR	3-(4, 5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium (MTS) assay	-	1, 3, 7, 14 days	MTA Fillapex showed significantly greater cytotoxicity compared to the control; On days 3, 7 and 14, MTA Fillapex was more toxic than iRoot SP; On day 3, AH Plus JET was more toxic than iRoot SP A reduction on cell viability was observed on day 7 for iRoot SP	iRoot SP was less toxic than both MTA Fillapex and AH Plus JET; and AH Plus JET was less toxic than MTA Fillapex
Jing et al., 2019 (38)	C-Root; BioRoot RCS; AH Plus	hPDLSCs	hPDLSCs + medium	NR	Cell counting kit-8 (CCK-8) assay	-	1, 3, 5, 7 days	BioRoot RCS had the highest cell proliferation at days 1 to 5; AH Plus had the lowest proliferation rate at days 3 to 7	BioRoot RCS and C-Root were less cytotoxic than AH Plus
Jo et al., 2020 (39)	Well-Root ST; AH Plus; Nishika Canal Sealer BG; Endoseal MTA	hPDLSCs	AH Plus	1:1; 1:2; 1:4	CCK-8 assay	-	3, 7, 14 days	Bioceramic sealers showed higher cell viability at all time periods compared to AH Plus	Bioceramic sealer showed the greatest cytocompatibility

Jung et al., 2018 (26)	BioRoot RCS; AH Plus; MTA Fillapex; Pulp Canal Sealer	Primary human osteoblasts (hOB)	hOB + medium	1:1; 1:2; 1:10	Living-cell-count; MTT-assay; Living/ dead-staining; LDH-assay	-	1, 7, 14, 21 days	Freshly mixed AH-Plus was cytotoxic, but it was not cytotoxic when sealer was set; MTA Fillapex and Pulp Canal Sealer were cytotoxic in a fresh and set state; BioRoot-RCS had the lowest toxicity in both states	The bioceramic sealer showed the greatest cytocompatibility among the investigated sealers
Lee et al., 2019 (40)	EndoSequence BC Sealer; AH Plus; MTA Fillapex	Murine calvaria pre-osteoblast cell line (MC3T3-E1) cells	MC3T3-E1 cells + medium	1; 1/5; 1/10 1/50; 1/100	EZ-Cytox-enhanced cell viability assay	-	24hs	AH Plus showed a significant decrease in cell viability compared to control at all dilutions; MTA Fillapex and EndoSequence BC Sealer had a significant decrease in cell viability compared to control at high dilutions	Calcium silicate–based sealers showed strong cell viability compared with AH Plus
Lim et al., 2015 (41)	ProRoot; AH Plus; EndoSeal	MC3T3-E1 cells	NR	NR	MTT assay	-	1, 3, 7, 14 days	ProRoot showed significantly higher cell viability compared to the other sealers; Viability of EndoSeal was significantly higher than AH Plus	ProRoot and EndoSeal had greater biocompatibility than AH Plus
López-García et al., 2019 (42)	Bio-C Sealer; TotalFill BC Sealer; AH Plus	hPDLSCs	hPDLSCs + medium;	1; 1:2; 1:4	MTT assay	-	24, 48, 72hs	Bio-C Sealer and TotalFill BC Sealer were significantly less cytotoxic than AH Plus in all dilutions and experimental periods	Bio-C Sealer and TotalFill BC Sealer demonstrated better cytocompatibility than AH Plus
Loushine et al., 2011 (43)	EndoSequence BC Sealer; AH Plus	MC3T3-E1	Pulp Canal Sealer EWT (positive control); Teflon disks (negative control)	NR	MTT assay	-	0, 1, 2, 3, 4, 5, 6 weeks	All set sealers had severe cytotoxicity at 24 hours;	All sealers had some degree of cytotoxicity
Mann et al., 2022 (54)	EndoSequence BC Sealer HiFlow; EndoSequence BC Sealer; AH Plus	hPDLFC	DMEM medium; 0.1% sodium dodecyl sulfate	NR	XTT-based cell viability assay	-	24, 48hs	Calcium silicate sealers had higher cell viability than AH Plus, without significant differences between them	Calcium silicate sealers were less cytotoxic than AH Plus
Mestieri et al., 2020 (27)	EndoSequence BC Sealer; AH Plus MTA Fillapex	3T3 fibroblasts	DMEM medium	1:1; 1:2; 1:4	MTT assay	-	6, 24hs	AH Plus and MTA Fillapex were significantly cytotoxic at a 1:1 dilution; At 1:2 and 1:4 dilutions, all sealers were similar to control, and MTA Fillapex was more cytotoxic than EndoSequence BC Sealer	EndoSequence BC Sealer had a greater cytocompatibility among the tested sealers

								In fresh media, AH Plus showed the lowest cell viability in all periods;	
Oh et al., 2020 (44)	CeraSeal; EndoSeal TCS; AH Plus	hPDLSCs	hPDLSCs + medium;	NR	CCK-8 assay	-	1, 3, 7 days	In fresh media, calcium silicate-based sealers showed similar viability to control at days 1 and 3, whereas at day 7; Cell viability of CeraSeal significantly increased compared to control and EndoSeal TCS;	Calcium silicate-based sealers appear to be less cytotoxic than epoxy-resin based sealers
								In setting media, cell viability was not different between materials over all periods	
Rodriguez-Lozano et al., 2017 (28)	TotalFill BC Sealer; AH Plus; MTA Fillapex	hPDLSCs	hPDLSCs + staurosporine solution	1:2; 1:4; 1:8	MTT assay; DNA-specific fluorochrome Hoechst 33342	-	24, 48, 72hs	TotalFill BC Sealer had significantly higher cell proliferation compared with AH Plus and MTA Fillapex; TotalFill BC Sealer cytotoxicity was lower than those observed in AH Plus and MTA Fillapex eluates	TotalFill BC Sealer had a greater cytocompatibility than AH Plus and MTA Fillapex
Rodríguez-Lozano et al., 2020 (45)	EndoSequence BC Sealer HiFlow; EndoSequence BC Sealer; AH Plus	hPDLSCs	hPDLSCs + medium	1; 1:2; 1:4	MTT assay	-	24, 48,72hs	EndoSequence BC Sealer HiFlow and EndoSequence BC Sealer had no cytotoxic effects regardless of the dilutions and periods of time; AH Plus was associated to a significant decrease in cell viability at all time-periods and dilutions	EndoSequence BC Sealer HiFlow and EndoSequence BC Sealer were more cytocompatibil than AH Plus
Sanz et al., 2021 (29)	EndoSequence BC Sealer HiFlow; Bio-C Sealer ION+; AH Plus	Human periodontal ligament cells (hPDLCs)	hPDLCs + medium	1:1; 1:2; 1:4	MTT assay	-	24, 48, 72hs	EndoSequence BC Sealer HiFlow and Bio-C Sealer ION+ showed similar results to the control group; At 48hs, undiluted EndoSequence BC Sealer HiFlow showed a higher production than the control; AH Plus showed higher cytotoxicity among the tested sealers at all moments of evaluation	The investigated calcium silicate-based sealers showed greater cytocompatibility compared to AH Plus
Sanz et al., 2022 (53)	AH Plus Bioceramic; EndoSequence BC Sealer; AH Plus	hPDLSCs	hPDLCs + medium	1:1; 1:2; 1:4	MTT assay	-	24, 48, 72hs	AH Plus Bioceramic and EndoSequence BC Sealer Sealer had an adequate cell viability at all time-points, similar to the control group; AH Plus Bioceramic, at 1:1 dilution, had a lower cell viability compared to the control group; AH Plus had a lower cell viability compared to the control group at all time-points	AH Plus Bioceramic and EndoSequence BC Sealer Sealer had a higher cytocompatibility compared to AH Plus

Seo et al., 2019 (46)	EndoSequence BC Sealer Sealer; BioRoot RCS; AH Plus; Endoseal MTA	Human dental pulp stem cells (hDPSCs)	hDPSCs + medium	NR	MTT assay	-	0, 24, 48, 72, 120hs	There were no differences between EndoSequence BC Sealer Sealer, BioRoot RCS, Endoseal MTA, and control group in any experimental period; AH Plus showed the lowest cell viability after 72hs	Calcium silicate-based sealers showed greater cytocompatibility
Silva et al., 2017 (47)	EndoSequence BC Sealer Sealer; AH Plus; MTA Fillapex; EndoSeal	3T3 fibroblasts cells	Unfilled root canals;	NR	MTT assay	-	24hs	AH Plus, EndoSeal and EndoSequence BC Sealer had similar cell activity to the negative control group; MTA Fillapex had a significantly stronger cytotoxic effect	AH Plus, EndoSeal, and EndoSequence BC Sealer Sealer were cytocompatible
Siregar et al., 2019 (48)	iRoot SP; AH Plus; GuttaFlow Bioseal	Lymphocites	Blood samples without sealers	NR	-	γ-H2AX assay	1, 3, 7 days	AH Plus had highest expression of foci on day 1, without foci expression on days 3 and 7; GuttaFlow Bioseal had an increasing expression at all days; iRoot SP had the lowest expressions at all days;	The highest values of genotoxicity were found for AH Plus, and the lowest for iRoot SP
Só et al., 2022 (55)	Sealer Plus BC; AH Plus; MTA Fillapex	hPDLSCs	hPDLSCs + medium	1:10	MTT assay	MN test	CYTOTOXICITY 24, 48, 72hs GENOTOXICITY 24hs	CYTOTOXICITY Sealer Plus BC had the lowest cytotoxicity, followed by the control group, MTA Fillapex, and AH Plus, at all evaluated periods; GENOTOXICITY No differences were observed among groups	All sealers presented low genotoxicity, and Sealer Plus BC presented the lowest cytotoxicity
Taraslia et al., 2018 (30)	TotalFill BC Sealer; BioRoot RCS; AH Plus; MTA Fillapex; GuttaFlow 2; Roth's 801	hPDLCs	hPDLCs + medium	NR	Costar Transwell cell viability assay	-	0, 24hs	GuttaFlow 2 had the highest cell viability in all periods; MTA Fillapex, TotalFill BC Sealer and BioRoot RCS had higher cell viability than AH Plus and Roth's, except for freshly mixed MTA Fillapex and BioRoot RCS	GuttaFlow 2, TotalFill BC Sealer, BioRoot RCS, and MTA Fillapex had increased cell viability compared to AH Plus and Roth's 801
Vouzara et al., 2018 (31)	BioRoot RCS; SimpliSeal; MTA Fillapex	3T3 cells	3T3 cells + medium	1:1; 1:2	Modified staining sulforhodamine B assay	-	24, 72hs	BioRoot RCS was less cytotoxic than the other sealers at all tested extracts, concentrations, and times of measurement.	BioRoot RCS had the lowest cytotoxicity among the investigated sealers

Willershausen et al., 2011 (49)	EndoSequence BC Sealer; AH Plus JET GuttaFlow; Pulp Canal Sealer	hPDLFC	hPDLFC + medium;	NR	Alamar blue assay;	-	0, 1, 6, 24, 48, 72, 96hs	GuttaFlow and EndoSequence BC Sealer were relatively non cytotoxic, while Pulp Canal Sealer EWT and AH Plus JET caused a significant decrease of cell proliferation	EndoSequence BC Sealer and GuttaFlow were highly biocompatible
Zhou et al., 2015 (32)	EndoSequence BC Sealer; AH Plus; MTA Fillapex	Human gingival fibroblasts	Human gingival fibroblasts + medium	1:2; 1:8; 1:32; 1:128	Quantitative flow cytometry test	-	0, 1, 2, 3, 4 weeks	EndoSequence BC Sealer showed higher cell viability at all extract concentrations than extracts from freshly mixed AH Plus and fresh and set MTA Fillapex, especially at high extract concentrations (1:2 and 1:8 dilutions); Extracts from set MTA Fillapex of 2 weeks and older were more cytotoxic than extracts from freshly mixed and 1-week-old; With extract concentrations of 1:32 and lower, MTA Fillapex was non-cytotoxic; After setting, AH Plus was non-cytotoxic, and the fibroblast cells grew on set AH Plus equally as well as on BC Sealer	EndoSequence BC Sealer had greater cytocompatibility than MTA Fillapex and AH Plus
Zordan-Bronzel et al., 2021 (52)	Sealer Plus BC; TotalFill BC Sealer; AH Plus	Human osteoblast-like (Saos-2) cells	20% DMSO (positive control) Serum-free DMEM (negative control)	1:1; 1:2; 1:4; 1:8; 1:16; 1:32	MTT assay Neutral red assay	-	1, 3, 7 days	MTT ASSAY: Sealer Plus BC in the 1:1 and 1:2 dilutions had significantly lower cell viability compared to the other sealers and the negative control; NEUTRAL RED ASSAY: AH Plus, Sealer Plus BC and TotalFill BC Sealer had no cytotoxic effects on Saos-2 cells, compared to negative control; At days 1 and 7, Sealer Plus BC had a significantly greater cell viability than positive control	In general, sealers had a similar pattern of cytotoxic behavior, but Sealer Plus BC presented greater cell viability after 7 days

Zoufan et al., 2011 (50)	EndoSequence BC Sealer; AH Plus; GuttaFlow; Tubli-Seal	L929 mouse fibroblasts	L929 mouse fibroblasts + medium	NR	MTT assay	-	24, 72hs	Freshly mixed AH Plus was more cytotoxic than the other sealers, and Tubli-Seal had less cell viability than EndoSequence BC Sealer and GuttaFlow, without differences between the latter; For the set sealers, Tubli-Seal and AH Plus had less cell viability than EndoSequence BC Sealer and GuttaFlow, without differences between the latter	GuttaFlow and EndoSequence BC Sealer sealers have lower cytotoxicity than the AH Plus and Tubli-Seal sealers

However, the condition investigated (set sealers) does not necessarily reflect a clinical condition, as the contact between the sealers and the periapical tissues usually occurs through extrusion beyond the apical foramen at the time of obturation. In this moment, the sealer is still fresh, and at the same time, blood and lymphatic vessels are present in the living tissue, diluting the substances[55]. In the other study[47], a 3D cell culture and an *in vitro* root model was used and, according to the authors themselves, the direct application of their results to the clinic could be mitigated. This may be explained due to the reduced contact area of the root canal sealer with the cell culture when compared to 2D cell cultures, likely decreasing the[47] toxic effects of the root canal sealers.

Only five studies compared the genotoxicity of calcium silicate-based sealers with epoxy resin-based sealers[22,34,35,48,55]. Four studies concluded that calcium silicate sealers are less genotoxic than epoxy resin sealers[22,34,35,48], and one study did not report differences[55]. As reported by the authors themselves, the absence of statistical differences may be related to the dilutions used in the study. In this study, AH Plus was the investigated epoxy resin sealer and, when diluted, there may occur a decrease of the resinous compound present in the sealer composition, allowing the sealer to demonstrate a similar behavior to the calcium silicate-based sealer[12]. Nevertheless, when extrusion occurs, the sealer is initially presented in higher concentrations contacting the periapical tissues and, therefore, it can potentially present a greater genotoxic effect[22].

Table 4. Quality assessment and risk of bias.

Study	Description of Cell Lineage	Control Group	Description of the Method of Cell Culture Preparation	Description of Sealers/Extracts Preparation	Reproducibility of Experiments	Statistical Analysis Description	Overall Risk of Bias
Almeida et al., 2020 (33)	Yes	Yes	Yes	No	Yes	Yes	LOW
Alsubait et al., 2018 (23)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Candeiro et al., 2016 (34)	Yes	Yes	Yes	No	Yes	Yes	LOW
Colombo et al., 2018 (24)	Yes	Yes	Yes	No	No	No	MODERATE
Eldeniz et al., 2016 (35)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Erdogan et al., 2021 (22)	Yes	Yes	Yes	No	Yes	Yes	LOW
Ferreira et al.,2022 (51)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Gaudin et al., 2020 (25)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Giacomino et al., 2019 (36)	Yes	Yes	Yes	No	Yes	Yes	LOW
Güven et al., 2013 (37)	Yes	Yes	Yes	No	No	Yes	MODERATE
Jing et al., 2019 (38)	Yes	Yes	Yes	No	No	Yes	MODERATE
Jo et al., 2020 (39)	Yes	Yes	Yes	Yes	Yes	Yes	LOW

Jung et al., 2018 (26)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Lee et al., 2019 (40)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Lim et al., 2015 (41)	Yes	No	Yes	No	No	Yes	MODERATE
López-García et al., 2019 (42)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Loushine et al., 2011 (43)	Yes	Yes	Yes	No	Yes	Yes	LOW
Mann et al., 2022 (54)	Yes	Yes	Yes	No	Yes	Yes	LOW
Mestieri et al., 2020 (27)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Oh et al., 2020 (44)	Yes	Yes	Yes	No	Yes	Yes	LOW
Rodriguez-Lozano et al., 2017 (28)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Rodríguez-Lozano et al., 2020 (45)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Sanz et al., 2021 (29)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Sanz et al., 2022 (53)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Seo et al., 2019 (46)	Yes	Yes	Yes	No	Yes	Yes	LOW
Silva et al., 2017 (47)	Yes	Yes	Yes	No	No	Yes	MODERATE

Siregar et al., 2019 (48)	Yes	Yes	Yes	No	No	Yes	MODERATE
Só et al., 2022 (55)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Taraslia et al., 2018 (30)	Yes	Yes	Yes	No	Yes	Yes	LOW
Vouzara et al., 2018 (31)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Willershausen et al., 2011 (49)	Yes	Yes	Yes	No	Yes	Yes	LOW
Zhou et al., 2015 (32)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Zordan-Bronzel et al., 2021 (52)	Yes	Yes	Yes	Yes	Yes	Yes	LOW
Zoufan et al., 2011 (50)	Yes	Yes	Yes	No	Yes	Yes	LOW

So far, there is no specific tool to evaluate the risk of bias of *in vitro* studies. Therefore, a tool based on the methodology adopted by previous systematic reviews of *in vitro* studies was used[16,17]. In this systematic review, the following parameters were accessed to determine the risk of bias of the included studies: description of cell lineage, presence of a control group, description of the cell culture preparation, description of sealer/extracts preparation, reproducibility of the experiment, and description of statistical analysis.

Established cell lines ensure the reproducibility of intra laboratory results and allow for the comparison of interlaboratory results[57]. All of the included studies reported for this parameter. As for the presence of a control group, only one study did not present a control group[41]. A control group consists of elements that present characteristics that allows the individual analysis of the impact of the investigated variables[58], allowing the inference of results. The reproducibility of the experiments was considered as this allows the validation of the results[59]. Six studies did not present this parameter[24,37,38,41,47,48]. As for the description of the statistical analysis, only one of the studies did not present this parameter[24].

Statistical analysis is important because it favors the correct interpretation of data arising from the research[60], and for this reason, it was considered one of the parameters for the risk of bias analysis. The parameters "description of the cell culture preparation" and "description of sealer/extracts preparation" were considered as they allow the understanding of what was performed during the research and allow the reproduction of these experiments in future research[61]. Six studies did not report for "description of sealer/extracts preparation"[24,37,38,41,47,48].

The present systematic review presents some limitations. First, it was not established the impact of sealers on the success rate of endodontic treatment when in contact with the periapical tissues. Thus, future studies are needed to determine whether the lower cytotoxicity and genotoxicity of calcium silicate-based sealers can present more positive results compared to epoxy resin-based sealers in clinical outcomes.

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CONCLUSION

Based on the results of this systematic review, it is possible to conclude that calcium silicate-based sealers are less cytotoxic and genotoxic than epoxy resin-based sealers.

REFERENCES

1. Kaur A, Shah N, Logani A, Mishra N. Biotoxicity of commonly used root canal sealers: A meta-analysis. J Conserv Dent. 2015;18(2):83–8.

2. Lim M, Jung C, Shin DH, Cho YB, Song M. Calcium silicate-based root canal sealers: a literature review. Restor Dent Endod. 2020;45(3):e35.

3. Hommez GMG, Coppens CRM, De Moor RJG. Periapical health related to the quality of coronal restorations and root fillings. Int Endod J. 2002;35(8):680–9.

4. AL-Haddad A, Aziz ZACA. Bioceramic-based root canal sealers: A review. Int J Biomater. 2016;2016:1–10.

5. Costa FMS, Fernandes MH, de Medeiros SRB. Genotoxicity of root canal sealers: a literature review. Clin Oral Investig. 2020;24(10):3347–62.

6. Huang T-H, Yang J-J, Li H, Kao C-T. The biocompatibility evaluation of epoxy resin-based root canal sealers *in vitro*. Biomaterials. 2002;23(1):77–83.

7. Scarparo RK, Grecca FS, Fachin EVF. Analysis of tissue reactions to methacrylate resin-based, epoxy resin-based, and zinc oxide-eugenol endodontic sealers. J Endod. 2009;35(2):229–32.

8. Jafari F, Jafari S. Composition and physicochemical properties of calcium silicate based sealers: A review article. J Clin Exp Dent. 2017;9(10):e1249–55.

9. Ribeiro DA, Yujra VQ, De Moura CFG, Handan BA, Viana MB, Yamauchi LY, et al. Genotoxicity induced by dental materials: A comprehensive review. Anticancer Res. 2017;37(8)4017–24.

10. Donnermeyer D, Bürklein S, Dammaschke T, Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. Odontology. 2019;107(4):421–36.

11. Benetti F, Queiroz IOA, Oliveira PHC, Conti LC, Azuma MM, Oliveira SHP, et al. Cytotoxicity and biocompatibility of a new bioceramic endodontic sealer containing calcium hydroxide. Braz Oral Res. 2019;33:e042.

12. Bin CV, Valera MC, Camargo SEA, Rabelo SB, Silva GO, Balducci I, et al. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. J Endod 2012;38(4):495–500.

13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann T, Mulrow CD, et al. Mapping of reporting guidance for systematic reviews and meta-analyses generated a comprehensive item bank for future reporting guidelines. J Clin Epidemiol. 2020;118:60–8.

14. Maia L, Antonio A. Systematic Reviews in Dental Research. A Guideline. J Clin Pediatr Dent. 2012;37(2):117–24.

15. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

16. Gorman CM, Ray NJ, Burke FM. The effect of endodontic access on all-ceramic crowns: A systematic review of in vitro studies. J. Dent. 2016;53:22–9.

17. Silva EJNL, Rover G, Belladonna FG, De-Deus G, Teixeira CT, Fidalgo TKS. Impact of contracted endodontic cavities on fracture resistance of endodontically treated teeth: a systematic review of in vitro studies. Clin Oral Investig. 2018;22(1):109–118.

18. Silva ECA, Tanomaru-Filho M, da Silva GF, Delfino MM, Cerri PS, Guerreiro-Tanomaru JM. Biocompatibility and bioactive potential of new calcium silicate-based endodontic sealers: Bio-C Sealer and Sealer Plus BC. J Endod. 2020;46(10)1470–7.

19. Zordan-Bronzel CL, Tanomaru-Filho M, Rodrigues EM, Chávez-Andrade GM, Faria G, Guerreiro-Tanomaru JM. Cytocompatibility, bioactive potential and antimicrobial activity of an experimental calcium silicate-based endodontic sealer. Int Endod J. 2019;52(7):979–986.

20. Bryan TE, Khechen K, Brackett MG, Messer RLW, El-Awady A, Primus CM, et al. In vitro osteogenic potential of an experimental calcium silicate-based root canal sealer. J Endod. 2010;36(7):1163–9.

21. Lee JK, Kim S, Lee S, Kim HC, Kim E. In vitro comparison of biocompatibility of calcium silicate-based root canal sealers. Mater (Basel, Switzerland). 2019;12(15):1–12.

22. Erdogan H, Yildirim S, Cobankara FK. Cytotoxicity and genotoxicity of salicylate- and calcium silicate-based root canal sealers on primer human periodontal ligament fibroblasts. Aust Endod J. 2021;47(3)645–653.

23. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of different concentrations of three root canal sealers on human mesenchymal stem cells. Biomolecules. 2018;8(3):1-7.

24. Colombo M, Poggio C, Dagna A, Meravini MV, Riva P, Trovati F, et al. Biological and physico-chemical properties of new root canal sealers. J Clin Exp Dent. 2018;10(3):e120–6.

25. Gaudin A, Tolar M, Peters OA. Cytokine production and cytotoxicity of calcium silicate–based sealers in 2- and 3-dimensional cell culture models. J Endod. 2020;46(6):818–826.

26. Jung S, Sielker S, Hanisch MR, Libricht V, Schäfer E, Dammaschke T. Cytotoxic effects of four different root canal sealers on human osteoblasts. PLoS One. 2018;13(3):e0194467.

27. Mestieri LB, Zaccara IM, Pinheiro LS, Barletta FB, Kopper PMP, Grecca FS. Cytocompatibility and cell proliferation evaluation of calcium phosphate-based root canal sealers. Restor Dent Endod. 2020;45(1):e2.

28. Rodríguez-Lozano FJ, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Forner L, Moradela JM. Evaluation of cytocompatibility of calcium silicate-based endodontic sealers and their effects on the biological responses of mesenchymal dental stem cells. Int Endod J. 2017;50(1):67–76.

29. Sanz JL, López-García S, Lozano A, Pecci-Lloret MP, Llena C, Guerrero-Gironés J, et al. Microstructural composition, ion release, and bioactive potential of new premixed calcium silicate-based endodontic sealers indicated for warm vertical compaction technique. Clin Oral Investig. 2021;25(3):1451–1462.

30. Taraslia V, Anastasiadou E, Lignou C, Keratiotis G, Agrafioti A, Kontakiotis EG. Assessment of cell viability in four novel endodontic sealers. Eur J Dent. 2018;12(2):287–391.

31. Vouzara T, Dimosiari G, Koulaouzidou EA, Economides N. Cytotoxicity of a new calcium silicate endodontic sealer. J Endod. 2018;44(5):849–852.

32. Zhou HM, Du TF, Shen Y, Wang Z-J, Zheng Y-F, Haapasalo M. In vitro cytotoxicity of calcium silicate-containing endodontic sealers. J Endod. 2015;41(1):56–61.

33. Almeida MM, Rodrigues CT, Matos AA, Carvalho KKT, Silva EJNL, Duarte MAH, et al. Analysis of the physicochemical properties, cytotoxicity and volumetric changes of AH Plus, MTA Fillapex and TotalFill BC Sealer. J Clin Exp Dent. 2020;12(11):e1058–e1065.

34. Candeiro GTMM, Moura-Netto C, D'Almeida-Couto RS, Azambuja-Júnior N, Marques MM, Cai S, et al. Cytotoxicity, genotoxicity and antibacterial effectiveness of a bioceramic endodontic sealer. Int Endod J. 2016;49(9):858–864.

35. Eldeniz AU, Shehata M, Högg C, Reichl FX. DNA double-strand breaks caused by new and contemporary endodontic sealers. Int Endod J. 2016;49(12):1141–1151.

36. Giacomino CM, Wealleans JA, Kuhn N, Diogenes A. Comparative biocompatibility and osteogenic potential of two bioceramic sealers. J Endod. 2019;45(1):51–6.

37. Güven EP, Yalvaç ME, Kayahan MB, Sunay H, Şahİn F, Bayirli G. Human tooth germ stem cell response to calcium-silicate based endodontic cements. J Appl Oral Sci. 2013;21(4):351–7.

38. Jing Y, Gong T, Duan C, Wang H, Zhang C, Neelakantan P. In vitro

cytocompatibility and osteogenic potential of calcium silicate-based dental cements in a root canal-filling model. J Int Med Res. 2019;48(4):300060519894801.

39. Jo SB, Kim HK, Lee HN, Kim YJ, Patel KD, Knowles JC, et al. Physical properties and biofunctionalities of bioactive root canal sealers in vitro. Nanomater (Basel, Switzerland). 2020;1(9):01-19.

40. Lee BN, Hong JU, Kim SM, Jang JH, Chang HS, Hwang YC, et al. Anti-inflammatory and osteogenic effects of calcium silicate–based root canal sealers. J Endod. 2019;45(1):73–8.

41. Lim ES, Park YB, Kwon YS, Shon WJ, Lee KW, Min KS. Physical properties and biocompatibility of an injectable calcium-silicate-based root canal sealer: in vitro and in vivo study. BMC Oral Health. 2015;15(1):1-7.

42. López-García S, Pecci-Lloret MR, Guerrero-Gironés J, Pecci-Lloret MP, Lozano A, Llena C, et al. Comparative cytocompatibility and mineralization potential of Bio-C Sealer and TotalFill BC Sealer. Mater (Basel, Switzerland). 2019;12(19):1–12.

43. Loushine BA, Bryan TE, Looney SW, Gillen BM, Loushine RJ, Weller RN, et al. Setting properties and cytotoxicity evaluation of a premixed bioceramic root canal sealer. J Endod. 2011;37(5):673–7.

44. Oh H, Kim EEE, Lee S, Park S, Chen D, Shin SJ, et al. Comparison of biocompatibility of calcium silicate-based sealers and epoxy resin-based sealer on human periodontal ligament stem cells. Mater (Basel, Switzerland). 2020;13(22)1-14.

45. Rodríguez-Lozano FJ, López-García S, García-Bernal D, Tomás-Catala CJ, Santos JM, Llena C, et al. Chemical composition and bioactivity potential of the new EndoSequence BC Sealer formulation HiFlow. Int Endod J. 2020;53(9):1216–1228.

46. Seo D-GG, Lee D, Kim Y-M, Song D, Kim S-Y. Biocompatibility and mineralization activity of three calcium silicate-based root canal sealers compared to conventional resin-based sealer in human dental pulp stem cells. Mater (Basel, Switzerland). 2019;12(15)1–12.

47. Silva EJNL, Zaia AA, Peters OA. Cytocompatibility of calcium silicate-based sealers in a three-dimensional cell culture model. Clin Oral Investig. 2017;21(5):1531–6.

48. Siregar I, Permitasari R, Kamizar, Margono A. Comparison of the potential genotoxicities of resin-, silicone-, and bioceramic-based root canal sealers against human lymphocytes. J Int Dent Med Res. 2019;12(1):88–94.

49. Willershausen I, Callaway A, Briseño B, Willershausen B. In vitro analysis of the cytotoxicity 1and the antimicrobial effect of four endodontic sealers. Head Face Med. 2011;7:1-9.

50. Zoufan K, Jiang J, Komabayashi T, Wang YH, Safavi KE, Zhu Q. Cytotoxicity evaluation of Gutta Flow and Endo Sequence BC sealers. Oral Surgery Oral Med Oral Pathol Oral Radiol Endodontology. 2011;112(5)657–661.

51. Ferreira GC, Pinheiro LS, Nunes JS, Mendes RA, Schuster CD, Soares RG, et al. Evaluation of the biological and physicochemical properties of calcium silicate-based and epoxy resin-based root canal sealers. J Biomed Mater Res. 2022;110(6):1344-1353.

52. Zordan-Bronzel CL, Tanomaru-Filho M, Torres FFE, Chávez-Andrade GM, Rodrigues EM, Guerreiro-Tanomaru JM. Physicochemical properties, cytocompatibility and antibiofilm activity of a new calcium silicate sealer. Braz Dent J. 2021;32(4):8-18.

53. Sanz JL, López-Garcia S, Rodríguez-Lozano FJ, Melo M, Lozano A, Llena C, et al. Cytocompatibility and bioactive potential of AH Plus Bioceramic Sealer: An in vitro study. Int Endod J. 2022;55(10):1066-1080.

54. Mann A, Zeng Y, Kirkpatrick T, van der Hooven R, Silva R, Letra A, et al. Evaluation of the physicochemical and biological properties of EndoSequence BC Sealer Sealer HiFlow. J Endod. 2022;48(1):123-131.

55. Só BB, Martins MD, Só MVR, Weissheimer T, Marques MM, Moreira MS. Genotoxicity and cytotoxicity comparison of calcium silicate-based and resin-based sealers on human periodontal ligament stem cells. Eur Endod J. 2022;7(2):129-134.

56. Sanz JL, Guerrero-Gironés J, Pecci-Lloret MP, Pecci-Lloret MR, Melo M. Biological interactions between calcium silicate-based endodontic biomaterials and periodontal ligament stem cells: A systematic review of in vitro studies. Int Endod J. 2021;54(11):2025-2043.

57. Pizzoferrato A, Ciapetti G, Stea S, Cenni E, Arciola C, Granchi D, et al. Cell culture methods for testing biocompatibility. Clin Mater. 1994;15(3)173-190.

58. Pithon MM. Importance of the control group in scientific research. Dental Press J Orthod. 2013;18(6):13–4.

59. Allison DB, Shiffrin RM, Stodden V. Reproducibility of research: Issues and proposed remedies. Proc Natl Acad Sci USA. 2018;115(11):2561–2.

60. Rodrigues CFS, Lima FJC, Barbosa FT. Importance of using basic statistics adequately in clinical research. Braz J Anesthesiol. 2017;67(6):619–625.

61. Faggion CM. Guidelines for reporting pre-clinical in vitro studies on dental materials. J Evid Based Dent Pract. 2012;12(4):182–9.