Systematic Review

Premixed calcium silicate-based root canal sealers have better biological properties than AH Plus: A systematic review and meta-analysis of *in vivo* animal studies and *in vitro* laboratory studies

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Abstract

Objectives: The aim was to determine whether premixed calcium silicate-based root canal sealers have better biological properties than AH Plus.

Materials and Methods: Searches of studies published up to January 2023 were performed in the PubMed/MEDLINE and EMBASE and via other methods (databases of the International Endodontic Journal, Journal of Endodontics, and gray literature). The inclusion criteria were *in vivo* animal and *in vitro* studies that analyzed the response in the dorsal subcutaneous tissue of rats, cell viability, and genotoxicity. Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias (RoB) tool for *in vivo* studies and modified CONSORT checklist for *in vitro* were appraised. Meta-analysis was performed using the Stata.

Results: Fifty-two studies were included. In the RoB, *in vivo* studies fulfilled 20%–50% of the items and *in vitro* 60%–100%. The studies included in the meta-analysis demonstrated better histocompatibility with the premixed calcium silicate-based sealers at 30 days and greater cell viability with these sealers when used in undiluted extracts in experimental period of 72 h and in extracts with 1:2 and 1:4 dilution in 24 and 72 h. In contrast, no difference between materials was found concerning genotoxicity.

Conclusion: Premixed calcium silicate-based root canal sealers have better histocompatibility and are less cytotoxic than the epoxy resin-based sealer AH Plus, demonstrating favorable biological behavior.

Keywords: Biocompatibility; calcium silicate; endodontic; epoxy resin; root canal filling materials; systematic review

INTRODUCTION

Successful endodontic treatment depends on adequate cleaning, shaping and filling of the root canal system. Obturation is the final operative step of this therapy and one of the most important.[1] The ideal filling material should have specific properties, such as excellent sealing, slow curing to ensure sufficient working time, absence of dimensional changes after curing, adequate adhesion to the root canal walls, radiopacity, no discoloring potential, solubility to solvents, insolubility to oral and tissue fluids, antimicrobial activity, tissue tolerance, and biocompatibility.[2] Different root canal sealers are currently available on the market and new materials are constantly

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being developed in an attempt to furnish all these favorable characteristics in a single product.[3]

Commercially available root canal sealers are categorized based on their chemical components.[4] AH Plus (Dentsply DeTrey) is an epoxy resin-based sealer that is considered the "gold standard" and is widely used as a comparison material due to its excellent physicochemical properties.[5] However, the biological behavior is also of extreme importance, as filling materials can be in direct contact with periapical tissues for extended periods[6] and can affect the success of endodontic treatment.[7,8] In this context, contemporary bioceramic sealers that are potentially bioactive have been developed and have received considerable attention in endodontics.[3]

Several studies have been conducted with premixed calcium silicate-based filling materials, demonstrating good physicochemical[9-12] and biological[4,13-16] properties. Furthermore, literature reviews have compared bioceramic sealers to AH Plus.[5,17,18] A systematic review by Silva Almeida *et al*.[17] was the first to compare physicochemical and biological properties globally between premixed calcium silicate-based endodontic sealers and conventional root canal filling materials. However, the meta-analysis was considered inappropriate because considerable heterogeneity was present in the selected studies. The meta-analysis of the studies by Silva *et al*.[5] and Silva *et al*.[18] demonstrated superior physicochemical properties (solubility and bond strength) of AH Plus in comparison with premixed calcium silicate-based root canal sealer. Nonetheless, biocompatibility has already been pointed out as a strong point of bioceramics by the qualitative synthesis of studies by Silva Almeida *et al*.,[17] Sanz *et al*.,[19] and Donnermeyer *et al*.[20] However, there is still no meta-analysis in the literature comparing the biological properties of premixed sealers based on calcium silicate with the current "gold standard." Therefore, the present study aimed to answer the following question: "Based on the results of *in vivo* animal studies and *in vitro* laboratory studies, do premixed calcium silicate-based root canal sealers have a better response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate), cell viability, and genotoxicity than AH Plus?" The hypothesis tested is that premixed calcium silicate-based root canal sealers have better biological properties than AH Plus.

MATERIALS AND METHODS

Protocol and registration

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020),[21] and the protocol was registered at the International Prospective Register of Systematic Reviews[22] under the code CRD42022275979. Considering the nonclinical nature of the investigation (systematic review of laboratory studies), the research question was adapted from the PICO framework: [23] Population (P) – *in vivo* animal models and *in vitro* cellular models; Intervention (I) – premixed calcium silicate-based root canal sealers; Comparison (C) – conventional AH Plus sealer (gold standard); and Outcomes (O) – biological properties (response in the dorsal subcutaneous tissue of rats – inflammatory infiltrate, cell viability, and genotoxicity).

Search strategy

Electronic searches were performed in the PubMed/MEDLINE and EMBASE databases for potentially eligible studies. "MeSH," "Emtree," and free terms were used in different combinations in the PubMed database. The MeSH terms were "Root Canal Filling Materials," "Epoxy Resins," "Materials Testing," "Subcutaneous Tissue," "Cytotoxicity Tests, Immunologic," "Cell Culture Techniques," "Stem Cells," and "Mutagenicity Tests" combined with the Boolean operators "OR" and "AND." This search was adapted in EMBASE with Emtree terms and automatic synonyms. To increase the yield of relevant studies, databases of the International Endodontic Journal and the Journal of Endodontics were checked. Gray literature was also searched (https://opengrey.eu/; https://scholar.google.com/; https://www.proquest.com/). No filters, limits, language, or publication date restrictions were applied. All searches were conducted from the earliest date available until January 2023. The search strategies for the different databases and platforms are displayed in Supplementary Table 1.

Study selection

Two independent reviewers (C.P.M and S.S.S.) first examined the titles of all studies retrieved from the databases. If a title indicated the possibility of inclusion, the abstract was analyzed and potentially eligible articles were then submitted to full-text analysis. Divergences of opinion regarding the inclusion/exclusion of any studies were resolved by discussion with a third reviewer (R.D.M.). Next, reference management was performed with the aid of the Rayyan program (https://rayyan.ai/mobile). Finally, the reference lists of all eligible studies were also hand-searched in an attempt to find additional studies not retrieved during the electronic search.

Eligibility criteria

The inclusion criteria for this review were *in vivo* animal studies and *in vitro* laboratory studies that investigated the biological properties of premixed calcium silicate-based root canal sealers in comparison to AH Plus. The biological properties of interest were the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate), cell viability, and genotoxicity. *In silico* studies, clinical trials, cohort studies, and case–control studies were excluded. Studies that evaluated other biological properties (e.g. antimicrobial effect, bioactivity, cellular

migration, cellular morphology, cell adhesion, and activity of inflammatory biomarkers), studies involving nonpremixed calcium silicate-based root canal sealers, studies with a comparator other than AH Plus, and studies that only assessed experimentally modified premixed calcium silicate-based root canal sealers were also excluded.

Data extraction

Two reviewers (C.P.M and S.S.S.) independently extracted data from the studies included in the review. Divergences of opinion were resolved by a third reviewer (R.D.M.). Articles were grouped according to the property tested. The following data were extracted: authors and year of publication, type of study (*in vivo* or *in vitro*), root canal sealers tested, type of animal or cell used, sample size, analysis method, experimental period, results (mean and standard deviation values), and conclusion. The WebPlotDigitizer tool[24] was used to extract mean and standard deviation values from the figures of studies that presented results in the form of graphs. The authors of the primary studies were contacted in cases of missing data.

Risk of bias assessment

Two independent reviewers (C.P.M and S.S.S.) appraised the methodological quality of the studies by assessing the risk of bias. Divergences of opinion regarding the inclusion/exclusion of any studies were resolved by discussion with a third reviewer (R.D.M.). The Risk of Bias (RoB) tool of the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)[25] was used for *in vivo* studies, which is an adapted version of the Cochrane RoB tool. In addition, the "Modified CONSORT checklist of items for reporting *in vitro* studies of dental materials" was applied for *in vitro* studies.[26] Finally, the studies were individually assessed regarding the fulfillment/nonfulfillment of each item of the quality appraisal instruments. The percentage of fulfilled items was then calculated (number of items fulfilled/total number of items × 100).

Data analysis

Microsoft Office Excel 2019® (Microsoft; Redmond, WA, USA) was used to enter the data and synthesize the results. Missing data were obtained through contact with the authors via E-mail, but occurred unsuccessful contact with one study[27] and thus excluded from the meta-analysis.

A separate meta-analysis was performed for each outcome (response in the dorsal subcutaneous tissue of rats – inflammatory infiltrate, cell viability, and genotoxicity). For the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate), the meta-analysis integrated the results of studies that implanted polyethylene tubes in the dorsal subcutaneous tissue of rats with an experimental period of seven (three studies) and/or 30 days (three studies), the results of which were expressed as scores (0: none or few inflammatory cells and no reaction; 1: <25 cells and mild reaction; 2: 25–125 cells and moderate reaction; and 3: ≥125 cells and severe reaction), and the mean and standard deviation of these values were obtained. For cell viability, the quantitative analysis encompassed the results of studies employing the two-dimensional 3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) as the analysis method with an experimental period of 24 and/or 72 h and extracts of root canal sealers in the final curing condition – undiluted (eight studies), dilution of 1:2 (five studies) and/or 1:4 (five studies). The results were expressed as mean and standard deviation of the cell proliferation rate (absorbance – proportional to the number of living cells). For genotoxicity, the meta-analysis was performed with three studies that used the micronucleus test (MNT) with an experimental period of 24 h. The results were expressed as mean and standard deviation of the number of cells with micronuclei for every 100 cells examined. The justification for not including studies in the meta-analysis is presented in Supplementary Table 2.

All analyses were performed using the Stata software, version 14.0 (Stata Corporation; College Station, TX, USA). For studies involving more than one premixed calcium silicate-based root canal sealer, the mean and standard deviation among the sealers were calculated. We used the following thresholds to assess I^2: 0%–40%: likely not important; 30%–60%: moderate heterogeneity; 50%–90%: substantial heterogeneity; and 75%–100%: considerable heterogeneity.[42] A random-effects model was employed in all analyses because the heterogeneity was considered high ($I^2 > 50\%$). Standardized mean differences (SMDs) between groups with 95% confidence intervals (CIs) constituted the effect size measure, since different measurements were used. Forest plots were created for all comparisons, and the results were presented as point estimates (SMD) with 95% CI.

Sensitivity analysis

Sensitivity analyses [Supplementary Figure 1] were used to determine whether an individual study significantly affected the pooled results (the "leave one out" approach) because the heterogeneity was considered high ($I^2 > 50\%$).

RESULTS

Study selection

The results of the electronic search, screening, and article selection process are presented in flowchart in Figure 1, according to the PRISMA 2020 instructions. The searches of the databases led to the retrieval of 5912 records, 1928 of which were duplicates and thus were manually removed. The screening of the titles and abstracts resulted in 67 potentially eligible articles. However, 20 articles were excluded after the full-text analysis – 18 involving nonpremixed calcium silicate-based root canal sealers and two involving an experimentally modified root canal sealer. Five additional articles were found via other methods.

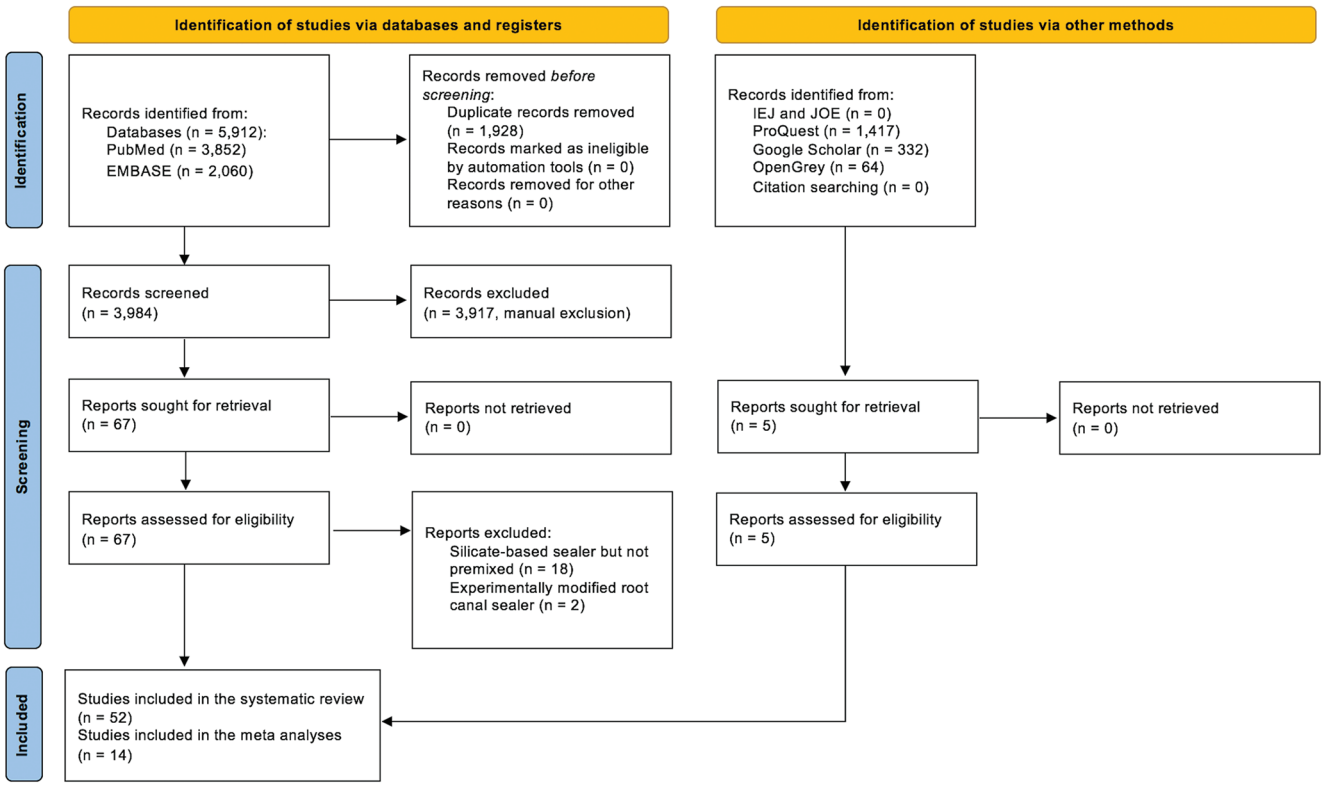

Figure 1: Systematic flowchart representing the study selection process. Based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 flow diagram[19]

Thus, 52 articles were included in the present systematic review and processed for data extraction.

Characteristics of the included studies

Table 1 displays the composition of the root canal sealers tested in the included studies and their manufacturers. Tables 2-4 show the data extracted from these studies, including the methods used to assess the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate), cell viability, and genotoxicity of the premixed calcium silicate-based root canal sealers in comparison to AH Plus. Three of the six studies that analyzed the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate) also assessed cell viability.[13,39,49] Forty-one studies included in this systematic review investigated only cell viability.[1-3,9-12,14,15,27-35,37,38,40,41,43-48,50-53,55-57,60-63,65] In addition, four studies that investigated genotoxicity also analyzed cell viability[4,54,58,64] and one study investigated only genotoxicity.[66]

Risk of bias assessment

The methodological quality of the studies is displayed in Table 5 (*in vivo* studies) and Table 6 (*in vitro* studies). For the *in vivo* studies, the generation of the allocation sequence (Item 1), allocation concealment (Item 3), blinding of researchers and/or caregivers (Item 5), and random outcome assessment (Item 6) were either unclear or not reported in all articles. All studies treated incomplete outcome data adequately (Item 8) and were free of selective outcome reporting (Item 9). Four of the six studies described the baseline characteristics of the groups (Item 2), three reported the random housing of the animals (Item 4), and three mentioned the blinding of the assessors (Item 7). No article was free of other problems that could result in a high risk of bias (Item 10), such as a crossover design in which all animals received the same intervention order. The rate of fulfilled items ranged from 20% to 50%.

For the *in vitro* studies, only one study[66] did not present a structured summary (Item 1). Introduction (Items 2a and 2b), Methods (Items 3, 4, and 10), and Results (Item 11) sections were well structured in all articles. However, Items 5 – 9 (referring to the sample size calculation and randomization process) were not fulfilled in any of the studies. Thus, based on Sanz *et al*.,[19] these items were not included in the RoB calculation. Potential limitations (Item 12) were addressed in 30 of the 49 studies. Funding sources (Item 13) were not reported in seven studies and only the studies by Martorano *et al*.[65] and Sanz *et al*.[37] mentioned an available protocol (Item 14). The rate of fulfilled items ranged from 60% to 100%.

Main findings – Response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate)

The comparison between premixed calcium silicate-based

Table 1: Composition of the tested materials and their manufacturers

Material	Manufacturer	Composition*
AH plus	Dentsply DeTrey	Component A: Epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide Component B: Adamantane amine, N, N-dibenzyl-5-oxanonane, TCD-diamine, calcium tungstate, zirconium oxide, silica
AH plus bioceramic sealer	Manufactured by Maruchi Distributed by Dentsply DeTrey	Zirconium dioxide, tricalcium silicate, dimethyl sulfoxide, lithium carbonate, thickening agent
Bio-C sealer	Angelus	Calcium silicates, calcium aluminate, calcium oxide, zirconium oxide, iron oxide, silicon dioxide, and dispersing agent
Bio-C sealer ION+	Angelus	Calcium silicate, magnesium silicate, polyethylene glycol, zirconium oxide, silicon dioxide nanoparticles, potassium sulfate, calcium sulfate hemihydrate
BrightEndo MTA sealer	GENOSS	Calcium silicates, zirconium oxide, bismuth oxide, solvent/thickening agent
Ceraseal	Meta Biomed Co.	Calcium silicates, zirconium oxide, thickening agent
Endoseal MTA	Maruchi	Calcium silicates, calcium aluminates, calcium sulfate, radiopacifier, thickening agent
Endoseal TCS	Maruchi	Tricalcium silicate, phyllosilicate mineral, zirconium oxide, dimethyl sulfoxide
EndoSequence BC sealer	Brasseler	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents
EndoSequence BC sealer HiFlow	Brasseler	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, fillers
iRoot SP	Innovative BioCeramix	Calcium silicate, calcium phosphate, calcium hydroxide, niobium oxide and zirconium oxide
Nano-ceramic sealer	B and L Biotech	Calcium silicates, zirconium oxide, filler, thickening agent
One-Fil	MEDICLUS	Calcium alumino silicate compound, zirconium oxide, hydrophilic polymer (thickening agent)
Sealer plus BC	MK Life	Calcium disilicate, nanoparticulate calcium trisilicate, and zirconium oxide
Sure-seal	Sure Dent Corporation	Calcium silicates, calcium phosphate, calcium hydroxide, filler, and thickening agents
TotalFill BC sealer	FKG Dentaire	Tricalcium silicate, dicalcium silicate, zirconium oxide, calcium hydroxide
TotalFill BC sealer HiFlow	FKG Dentaire	Zirconium oxide, tricalcium silicate, dicalcium silicate, calcium hydroxide, and fillers
Well-Root ST	Vericom	Calcium silicate compound, calcium sulfate dehydrate, calcium sodium phosphosilicate, zirconium oxide, titanium oxide, thickening agents

*Data extracted from the MSDS and/or provided by the manufacturer. MSDS: Material's safety data sheet

Table 2: Summary of parameters and results collected from the included studies for the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate)

Author, year	Study	Endodontic sealer	Animal or cell	Method	Period (days)	Conclusions
Lim *et al*., 2015[49]	*In vivo*	Endoseal MTA* AH Plus	Sprague Dawley rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	7	Inflammatory scores of Endoseal MTA were significantly lower than AH Plus
Zhang and Peng, 2015[59]	*In vivo*	iRoot SP* AH Plus	Wistar rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	7, 30, 60	At 30 days, AH Plus showed more infiltration of inflammatory cells than iRoot SP
Benetti *et al*., 2019[39]	*In vivo*	Sealer Plus BC* AH Plus	Wistar rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	7, 30	At 30 days, Sealer Plus BC was similar to the control and AH Plus exhibited greater inflammation than control
Alves Silva *et al*., 2020[6]	*In vivo*	Bio-C* Sealer Plus BC* AH Plus	Holtzman rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	7, 15, 30, 60	At 60 days, Bio-C and Sealer Plus BC showed no statistical difference between them and AH Plus presented the highest inflammatory cells values
Santos *et al*., 2021[16]	*In vivo*	TotalFill BC* TotalFill BC HiFlow* AH Plus	Wistar rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	8, 30	AH Plus showed the highest score for inflammation in both time periods
Ferreira *et al*., 2022[13]	*In vivo*	Sealer Plus BC* AH Plus	Wistar rats	Polyethylene tubes implanted in dorsal subcutaneous tissue	7, 30, 90	All sealers induced an initial inflammation reaction that decreased over time

*Premixed calcium silicate-based sealer. Experimental period was defined as days

sealers ($n = 26$) and AH Plus ($n = 26$) at 7 days demonstrated no significant difference between materials [Figure 2a], with a SMD and CI of −0.36 (−2.11, 1.39) and 82% heterogeneity among studies (I^2). Figure 2b displays the comparison between premixed calcium silicate-based sealers ($n = 28$) and AH Plus ($n = 28$) at 30 days, indicating better response in the dorsal subcutaneous tissue of rats of the bioceramic sealers, with a SMD and CI of −1.11 [−2.08, −0.15] and 59% heterogeneity among studies (I^2). Sensitivity analysis for the response in the dorsal subcutaneous tissue of rats at 7 days showed that the pooled estimate remained unchanged when removing any study. However, at 30 days, sensitivity analysis revealed that when removing one study,[59] the significance of the pooled estimate was lost (−1.34 [−3.13, 0.46]).

Main findings – Cell viability

Figure 3 shows the forest plots of the cell viability analysis comparing calcium silicate-based sealers and AH Plus. Figure 3a and b exhibits the comparison between premixed calcium silicate-based sealers ($n = 29$) and AH Plus ($n = 29$) for the analysis of cell viability using undiluted extracts

Table 3: Summary of parameters and results collected from the included studies for cell viability

Author, year	Study	Endodontic sealer	Animal or cell	Method	Period	Conclusions
Zhang *et al.*, 2010[50]	*In vitro*	iRoot SP* AH Plus	MG63 osteoblast-like cells	MTT assay	24 h	iRoot SP was noncytotoxic, whereas AH Plus was rated slightly cytotoxic
Zhang *et al.*, 2010[34]	*In vitro*	iRoot SP* AH Plus	L929 mouse fibroblasts	MTT assay	24 h	iRoot SP was noncytotoxic, whereas AH Plus was rated slightly cytotoxic
Loushine *et al.*, 2011[3]	*In vitro*	EndoSequence BC* AH Plus	MC3T3-E1 mouse osteoblast	MTT assay	24 h and 5 following weeks	Cytotoxicity of AH Plus gradually decreased over the 6-week and became noncytotoxic as early as the third week. EndoSequence BC remained moderately cytotoxic up to the fifth week and became mildly cytotoxic only at the sixth week
Willershausen *et al.*, 2011[28]	*In vitro*	EndoSequence BC* AH Plus	hPDLFC	Alamar Blue assay and ToxiLight BioAssay Kit	Alamar Blue: 0 h, 1 h, 6 h, 24 h, 48 h, 72 h, 96 h ToxiLight BioAssay: 24 h	Alamar Blue assay provided that AH Plus significantly inhibited cell growth compared to EndoSequence BC. With ToxiLight BioAssay, cells in contact with AH Plus showed a significantly higher cytotoxicity
Zoufan *et al.*, 2011[36]	*In vitro*	EndoSequence BC* AH Plus	L929 mouse fibroblasts	MTT assay	24 h, 72 h	AH Plus had less cell viability than EndoSequence BC
Güven *et al.*, 2013[33]	*In vitro*	iRoot SP* AH Plus	hTGSCs	MTS assay	24 h, 72 h, 7 d, 14 day	iRoot SP and AH Plus were similar in terms of the cytotoxicity parameters
Kim and Shin, 2014[45]	*In vitro*	EndoSeal MTA* AH Plus	MG63 osteoblast-like and HGF	WST-1 assay	24 h, 72 h, 7 days	EndoSeal MTA showed the lowest cytotoxicity against MG63 cells and HGF
Lim *et al.*, 2015[49]	*In vitro*	Endoseal MTA* AH Plus	MC3T3-E1 mouse osteoblast	MTT assay	24 h, 72 h, 7 days, 14 days	Viability of Endoseal MTA treated cells was significantly higher than AH Plus
Zhou *et al.*, 2015[53]	*In vitro*	EndoSequence BC* AH Plus	HGF	Quantitative flow cytometry	0 week, 1 week, 2 weeks, 3 weeks, 4 weeks	EndoSequence BC showed higher viabilities at all concentrations than AH Plus
Candeiro *et al.*, 2016[54]	*In vitro*	EndoSequence BC* AH Plus	HGF	MTT assay	24 h, 72 h, 5 days, 7 days	EndoSequence BC had significantly higher cell viability than AH Plus
Eldeniz *et al.*, 2016[58]	*In vitro*	iRoot SP* AH Plus	New PDL using lentiviral gene transfer hTERT	XTT assay	24 h	iRoot SP was the least cytotoxic sealer
Silva *et al.*, 2016[38]	*In vitro*	EndoSequence BC* AH Plus	Balb/c 3T3 fibroblasts cells	MTT assay	24 h	EndoSequence BC showed the lowest cytotoxicity
Rodríguez-Lozano *et al.*, 2017[60]	*In vitro*	TotalFill BC* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	TotalFill BC exhibited a higher cytocompatibility than AH Plus
da Silva *et al.*, 2017[47]	*In vitro*	EndoSeal MTA* EndoSequence BC* AH Plus	Balb/c 3T3 fibroblasts cells	MTT assay	24 h	EndoSeal MTA, EndoSequence BC and AH Plus showed cell viability that was similar to the negative control group
Alsubait *et al.*, 2018[1]	*In vitro*	EndoSequence BC* AH Plus	hMSCs	Alamar Blue assay	24 h, 72 h, 7 days	Cytotoxicity of EndoSequence BC was less than AH Plus
Kebudi Benezra *et al.*, 2018[43]	*In vitro*	Endoseal MTA* AH Plus	HGF	MTT assay	24 h	Both AH Plus and Endoseal MTA did not encourage cell growth on the material surface
Beshr and Abdelrahim, 2018[32]	*In vitro*	TotalFill BC* AH Plus	WI-38 cell line human	MTT assay	24 h, 72 h	AH Plus and TotalFill BC showed similar cytotoxicity
Colombo *et al.*, 2018[2]	*In vitro*	TotalFill BC* AH Plus	HGF	MTT assay	24 h, 48 h, 72 h	AH Plus had a moderate cytotoxicity. TotalFill BC showed no cytotoxic effect
Taraslia *et al.*, 2018[55]	*In vitro*	TotalFill BC* AH Plus	hPDLCs	Costar Transwell	72 h	TotalFill BC presented higher number of viable cells in comparison to the AH Plus
Benetti *et al.*, 2019[39]	*In vitro*	Sealer Plus BC* AH Plus	L929 mouse fibroblasts	Alamar Blue assay	24 h	A reduction in cell viability was observed in the extracts that were more diluted for Sealer Plus BC when compared to that of control and AH Plus
Giacomino *et al.*, 2019[51]	*In vitro*	EndoSequence BC* AH Plus	Murine osteoblast precursor cell line (IDG-SW3)	Luminescence assay based on adenosine triphosphate quantification	7 days	EndoSequence BC was less toxic to osteoblast precursor cells than AH Plus
Lee *et al.*, 2019[61]	*In vitro*	EndoSeal MTA* Nano-ceramic* Well-Root ST* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h, 7 days	AH Plus showed the lowest cell viability through all experimental periods among all of the tested sealers

Contd...

Table 3: Contd...

Author, year	Study	Endodontic sealer	Animal or cell	Method	Period	Conclusions
Lee *et al*., 2019[56]	*In vitro*	EndoSequence BC* AH Plus	MC3T3-E1 mouse osteoblast	WST-1	24 h	EndoSequence BC showed strong cell viability compared with AH Plus
López-García *et al*., 2019[46]	*In vitro*	Bio-C* TotalFill BC* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	TotalFill BC and Bio-C showed higher cell viability than AH Plus
Mestieri *et al*., 2020[62]	*In vitro*	EndoSequence BC* AH Plus	3T3 fibroblasts	MTT assay	6 h, 24 h	AH Plus revealed greater cytotoxicity at 1:1 dilution when compared to control. At 1:2 and 1:4 dilutions, all sealers were similar to control
Seo *et al*., 2019[11]	*In vitro*	EndoSequence BC* Endoseal MTA* AH Plus	hDPSCs	MTT assay	0 h, 24 h, 48 h, 72 h, 120 h	EndoSequence BC and Endoseal MTA showed superior cell viability compared to AH Plus
Souza *et al*., 2019[41]	*In vitro*	EndoSequence BC* AH Plus	Monocyt and PMNs	Annexin-V/ Propidium Iodide double stain using the FACSCalibur cytometer	4 h (PMNs); 24 h (monocyt)	AH Plus and EndoSequence BC resulted in a significant reduction in the percentage of viable cells compared with the control
Zordan-Bronzel *et al*., 2019[31]	*In vitro*	TotalFill BC* AH Plus	Human osteoblast-like cells, Saos-2	MTT and NR assays	24 h	MTT and NR revealed that AH Plus and TotalFill BC had no cytotoxic effects
Almeida *et al*., 2020[40]	*In vitro*	TotalFill BC* AH Plus	NIH3T3 murine fibroblasts	MTT assay	24 h, 48 h, 72 h	AH Plus showed higher cytotoxicity than TotalFill BC
Jo *et al*., 2020[9]	*In vitro*	Endoseal MTA* Well-Root ST* AH Plus	hPDLSCs	kit-8 (CCK-8)	6 h, 12 h, 24 h, 72 h	AH Plus showed a certain degree of cell toxicity, while the other sealers showed eminent cytocompatibility
Oh *et al*., 2020[15]	*In vitro*	CeraSeal* EndoSeal TCS* AH Plus	hPDLSCs	kit-8 (CCK-8)	24 h, 72 h, 7 days	In fresh media, AH Plus showed the lowest cell viability in all experimental periods. In setting media, cell viability was not significantly different between materials over all periods
Rodríguez-Lozano *et al*., 2020[52]	*In vitro*	EndoSequence BC HiFlow* EndoSequence BC* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	AH Plus group showed the lowest cell viability rates in comparison to the other experimental groups
Zheng *et al*., 2020[48]	*In vitro*	iRoot SP* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	iRoot SP was the least toxicity compared to AH Plus
Erdogan *et al*., 2021[4]	*In vitro*	iRoot SP* AH Plus	hPDLFC	XTT assay	0 h, 6 h, 12 h, 24 h, 48 h, 72 h	iRoot SP showed higher viability at all concentrations and times than AH Plus
Jun *et al*., 2021[44]	*In vitro*	Well-Root ST* AH Plus	MC3T3-E1 mouse osteoblast	WST-8 assay	24 h, 48 h	Well-Root ST showed higher viability at 48h than AH Plus
Park *et al*., 2021[12]	*In vitro*	BrightEndo MTA* CeraSeal* EndoSeal TCS* One-Fil* AH Plus	hPDLFC	MTT assay	Fresh extraction: 24 h, 48 h, 72 h, 7 days Setting extraction: 24 h, 72 h, 7 days	For the fresh extraction medium, the calcium silicate-based sealer had significantly higher number of living cells than AH Plus. For the setting extraction medium, AH Plus and the calcium silicate-based sealer showed a similar tendency
Saghiri *et al*., 2021[63]	*In vitro*	Sure-Seal* AH Plus	L929 mouse fibroblasts	MTS assay	24 h, 48 h, 72 h, 96 h	AH Plus showed higher cytotoxicity than other experimental group
Sanz *et al*., 2021[10]	*In vitro*	Bio-C ION+* EndoSequence BC HiFlow* AH Plus	hPDLCs	MTT assay	24 h, 48 h, 72 h	Bio-C ION+and EndoSequence BC HiFlow showed positive results in cytocompatibility assays, unlike AH Plus
Zordan-Bronzel *et al*., 2021[35]	*In vitro*	Sealer Plus BC* TotalFill BC* AH Plus	Human osteoblast-like cells, Saos-2	MTT and NR assays	24 h	In the MTT assay, Sealer Plus BC in the 1:1 and 1:2 dilutions had significantly lower cell viability. NR assay revealed that AH Plus, Sealer Plus BC and TotalFill BC had no cytotoxic effects
Ferreira *et al*., 2022[13]	*In vitro*	Sealer Plus BC* AH Plus	APCs	MTT and SRB assays	MTT assay: 24 h, 72 h SRB assay: 72 h	Sealer Plus BC had better results compared to AH Plus
Janini *et al*., 2022[27]	*In vitro*	Bio-C Sealer* TotalFill BC* AH Plus	Human osteoblast-like cells, Saos-2	MTT assay	24 h	AH Plus had the lowest cytotoxicity

Contd...

Table 3: Contd...

Author, year	Study	Endodontic sealer	Animal or cell	Method	Period	Conclusions
Mann *et al.*, 2022[14]	*In vitro*	EndoSequence BC* EndoSequence BC HiFlow* AH Plus	hPDLFC	XTT assay	24 h, 48 h	Cell viability was higher for EndoSequence BC HiFlow and EndoSequence BC than AH Plus
Sanz *et al.*, 2022[37]	*In vitro*	AH Plus Bioceramic* EndoSequence BC* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	AH Plus Bioceramic and EndoSequence BC exhibited a significantly higher cytocompatibility than the AH Plus
Sheela *et al.*, 2023[57]	*In vitro*	TotalFill BC* AH Plus	Human Osteoblast	XTT assay	24 h	At low concentrations, TotalFill BC showed higher viability cellular than AH Plus
Só *et al.*, 2022[64]	*In vitro*	Sealer Plus BC* AH Plus	hPDLSCs	MTT assay	24 h, 48 h, 72 h	Sealer Plus BC presented the lowest cytotoxicity
Wuersching *et al.*, 2022[29]	*In vitro*	TotalFill BC* AH Plus	hPDLFC and hMSCs	WST-8 assay	24 h, 7 days	AH Plus was severely cytotoxic to hPDLFC and hMSCs
Martorano *et al.*, 2023[65]	*In vitro*	Sealer Plus BC* AH Plus	Macrophage RAW 264.7 mouse cells	MTT assay and LIVE/DEA Cytotoxicity Kit	24 h, 48 h	Greater viability and mitochondrial activity were observed in cultures exposed to bioceramic sealer compared to AH Plus
Souza *et al.*, 2023[30]	*In vitro*	AH Plus Bioceramic* EndoSequence BC* AH Plus	hPDLFC	XTT assay	24 h, 48 h	AH Plus Bioceramic and EndoSequence BC showed significantly higher cell viability than AH Plus

*Premixed calcium silicate-based sealer. Experimental period was defined as hours, days, or weeks. PDL: Periodontal ligament, APCs: Apical papillary cells, hDPSCs: Human dental pulp stem cells, HGF: Human gingival fibroblast, hMSCs: Human mesenchymal stem cells, hPDLCs: Human PDL cells, hPDLFC: Human PDL fibroblast cell, hPDLSCs: Human PDL stem cells, hTERT: human telomerase reverse transcriptase, hTGSCs: Human tooth germ stem cells, MTS: (3-(4, 5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy- phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium), MTT: 3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide, NR: Neutral red, PMNs: Polymorphonuclears, SRB: Sulforhodamine B, XTT: (sodium 30-[1- (phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate), WST: Water-soluble tetrazolium salt

Table 4: Summary of parameters and results collected from included studies for genotoxicity

Author, year	Study	Endodontic sealer	Animal or cell	Method	Period	Conclusions
Candeiro *et al.*, 2016[54]	*In vitro*	EndoSequence BC* AH Plus	HGF	MNT	24 h	EndoSequence BC had a significantly smaller percentage of cells with micronucleus than AH Plus
Eldeniz *et al.*, 2016[58]	*In vitro*	iRoot SP* AH Plus	New PDL cell line using lentiviral gene transfer of hTERT	c-H2AX focus assay	6 h	iRoot SP demonstrated significantly more DNA double-strand breaks formation
Siregar *et al.*, 2019[66]	*In vitro*	iRoot SP* AH Plus	Lymphocyte human	γ-H2AX assay	24 h, 72 h, 7 days	The highest value of genotoxicity was found with AH Plus after incubation for one day, whereas the lowest genotoxicity was observed with iRoot SP after incubation for three and seven days
Erdogan *et al.*, 2021[4]	*In vitro*	iRoot SP* AH Plus	hPDLFC	MNT	24 h	Genotoxicity potential of AH Plus is high and iRoot SP has no genotoxic effect
Só *et al.*, 2022[64]	*In vitro*	Sealer Plus BC* AH Plus	hPDLSCs	MNT	24 h	All sealers presented low genotoxicity

*Premixed calcium silicate-based sealer. Experimental period was defined as hours, days. HGF: Human gingival fibroblast, PDL: Periodontal ligament, hPDLFC: Human PDL fibroblast cell, hPDLSCs: Human PDL stem cells, hTERT: Human telomerase reverse transcriptase, MNT: Micronucleus formation test

of root canal sealers. At 24 h [Figure 3a], no significant difference between materials was found, with a SMD and CI of 6.27 (−0.54, 13.09) and 75% heterogeneity among studies (I^2). At 72 h [Figure 3b], greater cell viability was found for the bioceramic root sealers, with a SMD and CI of 8.06 (0.20, 15.92) and 85% heterogeneity among studies (I^2). Sensitivity analysis for cell viability at 24 h using undiluted extracts of root canal sealers revealed that when removing two studies, a significant difference between materials was observed: omitting Seo *et al.*[11] – 9.05 (0.05, 18.05); omitting Park *et al.*[12] – 9.00 (0.65, 17.36). At 72 h, sensitivity analysis revealed that when removing five studies, the significance of the pooled estimate was lost: omitting Sanz *et al.*[10] – 5.62 (−0.86, 12.10); omitting Seo *et al.*[11] – 10.46 (−0.25, 21.17); omitting López-García *et al.*[46] – 5.30 (−1.01, 11.61); omitting Rodríguez-Lozano *et al.*[52] – 5.22 (−1.20, 11.64); omitting Sanz *et al.*[37] – 5.18 (−1.19, 11.55).

Figure 3c and d shows the comparison between premixed calcium silicate-based sealers ($n = 17$) and AH Plus ($n = 17$) for cell viability analysis using extracts of root canal sealers with 1:2 dilutions. Greater cell viability was found for the bioceramic root sealers in both experimental periods: 24 h [Figure 3c] and 72 h [Figure 3d], with a SMD and CI of 15.98 (3.55, 28.40) and 18.59 (4.36, 32.83), respectively, as well as 75% and 74% heterogeneity among studies, respectively (I^2). Sensitivity analysis for cell viability using

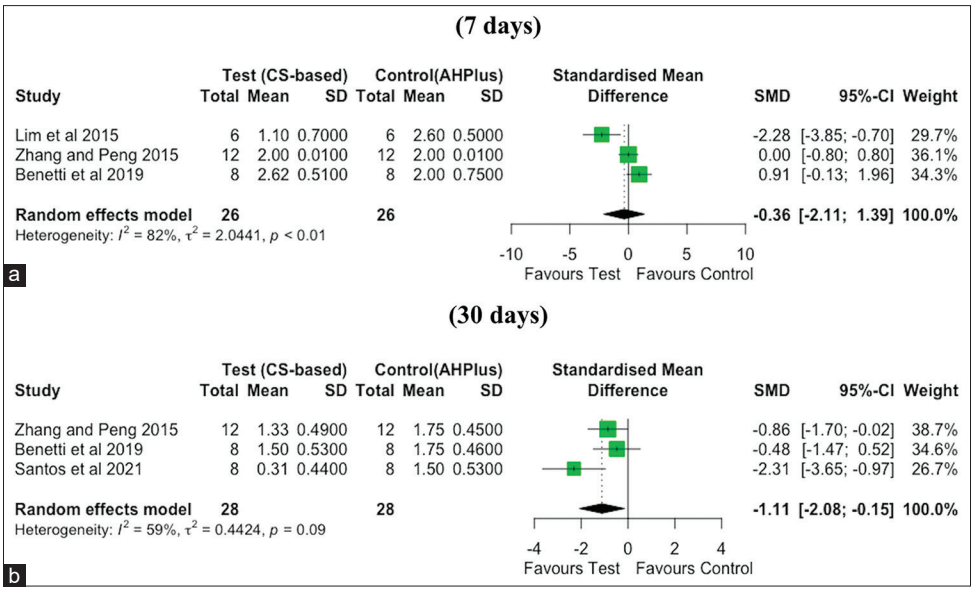

Figure 2: Forest plots of the analysis of the response in the dorsal subcutaneous tissue of rats (inflammatory infiltrate) comparing calcium silicate-based sealer and AH Plus. (a) Response in the dorsal subcutaneous tissue of rats - inflammatory infiltrate (7 days). (b) Response in the dorsal subcutaneous tissue of rats - inflammatory infiltrate (30 days) CI: Confidence interval, SMD: Standardized mean differences, SD: Standard deviation

Table 5: Quality assessment of the included *in vivo* studies

Studies	SYRCLE's RoB tool										
	1	2	3	4	5	6	7	8	9	10	%
Lim *et al*., 2015[49]	U	N	N	N	N	N	N	Y	Y	U	20
Zhang and Peng, 2015[59]	U	Y	N	Y	N	N	Y	Y	Y	U	50
Benetti *et al*., 2019[39]	N	Y	N	Y	N	N	N	Y	Y	N	40
Alves Silva *et al*., 2020[6]	N	N	N	N	N	N	N	Y	Y	N	20
Santos *et al*., 2021[16]	N	Y	N	Y	N	N	Y	Y	Y	N	50
Ferreira *et al*., 2022[13]	N	Y	N	N	N	N	Y	Y	Y	N	40

Based on the checklist of items from "SYRCLE's risk of bias tool for animal studies" U: Unclear, N: Not reported on the study, Y: Reported on the study, 1: Selection bias/sequence generation, 2: Selection bias/baseline characteristics, 3: Selection bias/allocation concealment, 4: Performance bias/random housing, 5: Performance bias/blinding, 6: Detection bias/random outcome assessment, 7: Detection bias/blinding, 8: Attrition bias/incomplete outcome data, 9: Reporting bias/selective outcome reporting, 10: Other/other sources of bias, %: Percentage of compliance per article

extracts of root canal sealers with 1:2 dilution showed that the pooled estimate remained unchanged when removing any study.

Figure 3e and f exhibits the forest plot comparing premixed calcium silicate-based sealers (n = 17) and AH Plus (n = 17) for cell viability analysis using extracts of root canal sealers with 1:4 dilutions. Again, greater cell viability was found for the bioceramic root sealers in both experimental periods: 24 h [Figure 3e] and 72 h [Figure 3f], with a SMD and CI of 7.32 (1.97, 12.66) and 16.10 (3.71, 28.49), respectively, as well as 68% heterogeneity among studies (I^2) in both analyses. Sensitivity analysis for cell viability using extracts of root canal sealers with 1:4 dilution showed that the pooled estimate remained unchanged when removing any study.

Main findings – Genotoxicity as indicated by micronucleus test

Finally, Figure 4 displays the comparison between premixed calcium silicate-based sealers (n = 10) and AH Plus (n = 10), demonstrating no significant difference between materials in terms of genotoxicity as indicated by MNT, with a SMD and CI of − 1.99 (−4.81, 0.83) and 69% heterogeneity among studies (I^2). Sensitivity analysis for genotoxicity as indicated by MNT showed that the pooled estimate remained unchanged when removing any study.

DISCUSSION

The biological properties of premixed calcium silicate-based root canal sealers have been widely described.[4,13-17] However, no previous meta-analysis has been conducted to integrate the results of these studies and test the hypothesis that premixed calcium silicate-based sealers have better biological properties than the "gold standard" AH Plus. The present study tested this alternative hypothesis and the results suggest that the new bioceramic sealers have better response in the dorsal subcutaneous tissue of rats and lower cytotoxicity than AH Plus, whereas no difference was found with regard to genotoxicity as indicated by MNT.

Biocompatibility analysis of endodontic materials is complex. Some argue that animal experiments are undoubtedly essential to biological testing.[67] Subcutaneous implantation in the connective tissue using an animal model is one of the most appropriate tests to determine the development of local reactions induced by endodontic

Table 6: Quality assessment of the included *in vitro* studies

Studies	Modified CONSORT checklist															
	1	2a	2b	3	4	5	6	7	8	9	10	11	12	13	14	%
Zhang *et al.*, 2010[50]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Zhang *et al.*, 2010b[34]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	N	N	70
Loushine *et al.*, 2011[3]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Willershausen *et al.*, 2011[28]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Zoufan *et al.*, 2011[36]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	N	N	70
Güven *et al.*, 2013[33]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	N	N	80
Kim and Shin, 2014[45]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	N	N	80
Lim *et al.*, 2015[49]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Zhou *et al.*, 2015[53]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Candeiro *et al.*, 2016[54]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Eldeniz *et al.*, 2016[58]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Silva *et al.*, 2016[38]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Rodríguez-Lozano *et al.*, 2017[60]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
da Silva *et al.*, 2017[47]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Alsubait *et al.*, 2018[1]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Kebudi Benezra *et al.*, 2018[43]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Beshr and Abdelrahim, 2018[32]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	N	N	80
Colombo *et al.*, 2018[2]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Siregar *et al.*, 2019[66]	N	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	N	N	60
Taraslia *et al.*, 2018[55]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Benetti *et al.*, 2019[39]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Giacomino *et al.*, 2019[51]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Lee *et al.*, 2019[61]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Lee *et al.*, 2019[56]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
López-García *et al.*, 2019[46]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Mestieri *et al.*, 2020[62]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Seo *et al.*, 2019[11]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Souza *et al.*, 2019[41]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Zordan-Bronzel *et al.*, 2019[31]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Almeida *et al.*, 2020[40]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Jo *et al.*, 2020[9]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Oh *et al.*, 2020[15]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Rodríguez-Lozano *et al.*, 2020[52]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Zheng *et al.*, 2020[48]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Erdogan *et al.*, 2021[4]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Jun *et al.*, 2021[44]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Park *et al.*, 2021[12]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Saghiri *et al.*, 2021[63]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Sanz *et al.*, 2021[10]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Zordan-Bronzel *et al.*, 2021[35]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Ferreira *et al.*, 2022[13]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Janini *et al.*, 2022[27]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	N	N	70
Mann *et al.*, 2022[14]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	N	Y	N	80
Sanz *et al.*, 2022[37]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	Y	100
Sheela *et al.*, 2023[57]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Só *et al.*, 2022[64]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Wuersching *et al.*, 2022[29]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90
Martorano *et al.*, 2023[65]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	Y	100
Souza *et al.*, 2023[30]	Y	Y	Y	Y	Y	NA	NA	NA	NA	NA	Y	Y	Y	Y	N	90

NA: Nonapplicable, N: Not reported on the study, Y: Reported on the study, 1: Abstract, 2a: Introduction/background, 2b: Introduction/objectives, 3: Methods/intervention, 4: Methods/outcomes, 5: Methods/sample size, 6: Methods/randomization sequence generation, 7: Methods/randomization allocation concealment mechanism, 8: Methods/implementation, 9: Methods/blinding, 10 Methods/statistical methods, 11: Results, 12: Discussion/limitation, 13: Other information/funding, 14: Other information/protocol, %: Percentage of compliance per article. Based on the checklist of items from "Guidelines for Reporting Preclinical *In Vitro* Studies on Dental Materials" (Faggion[26])

materials.[16,59] However, few studies included in the present systematic review used this method,[6,13,16,39,49,59] probably because such tests are more costly and time-consuming. Furthermore, it is not easy to control the numerous variables involved in the experiments and there are ethical aspects to consider.[67]

Rodents are the most widely used animals due to their low cost, high genetic homogeneity, and ease of handling.[67] All animal studies in the present review used rats, especially the Wistar line.[13,16,39,59] In addition, different implantation periods are needed for histological studies.[67] The periods selected for the meta-analysis (7 and 30 days) were consistent with most studies on tissue reactions to endodontic materials implanted in subcutaneous connective tissue[6,13,16,39,49,59] and respectively reflect the early and late inflammatory reactions.

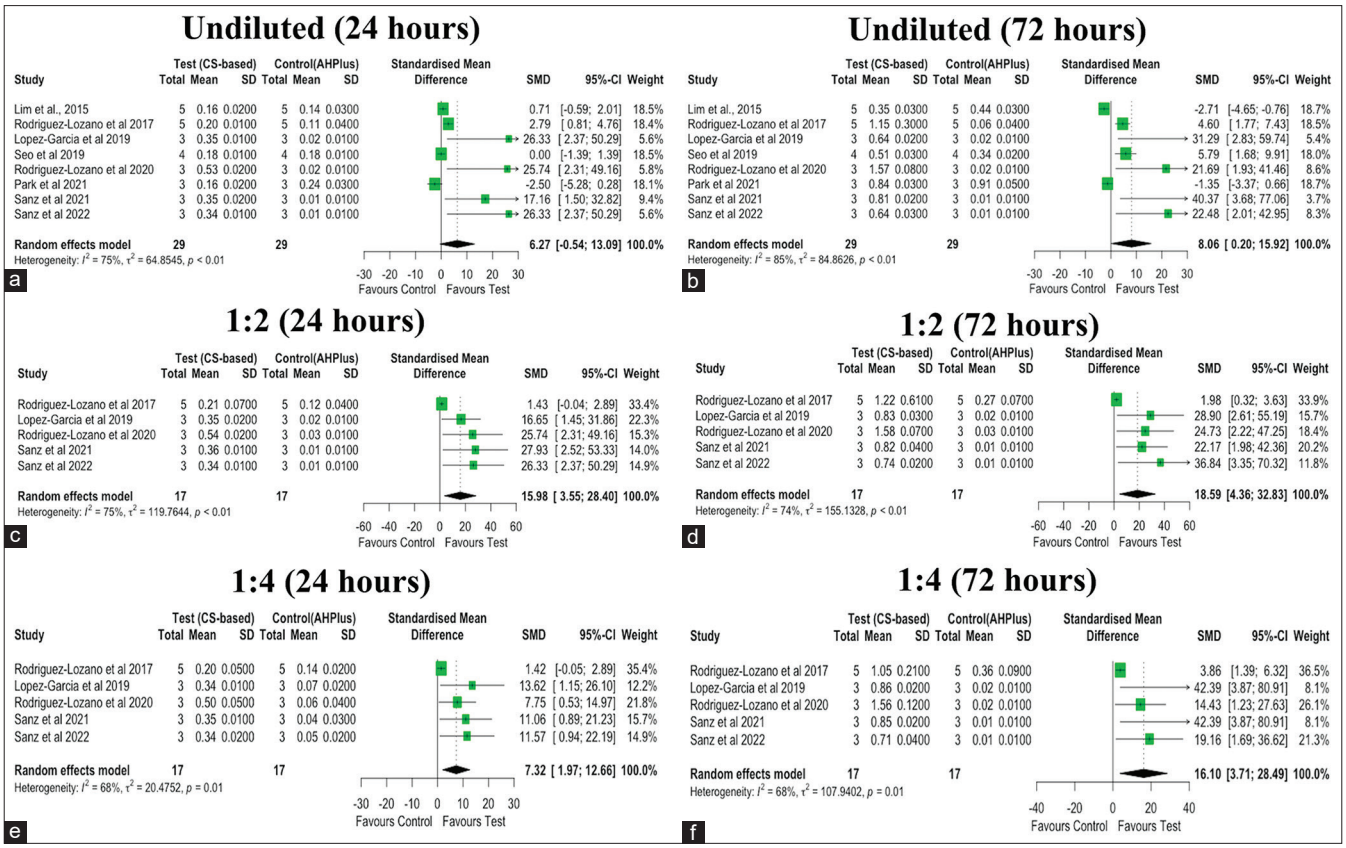

Figure 3: Forest plots of the analysis of cell viability comparing calcium silicate-based sealer and AH Plus. (a) Cell viability-endodontic sealer extracts undiluted (24 h). (b) Cell viability-endodontic sealer extracts undiluted (72 h). (c) Cell viability-endodontic sealer extracts with 1:2 dilutions (24 h). (d) Cell viability-endodontic sealer extracts with 1:2 dilutions (72 h). (e) Cell viability-endodontic sealer extracts with 1:4 dilutions (24 h). (f) Cell viability-endodontic sealer extracts with 1:4 dilutions (72 h). CI: Confidence interval, SMD: Standardized mean differences, SD: Standard deviation

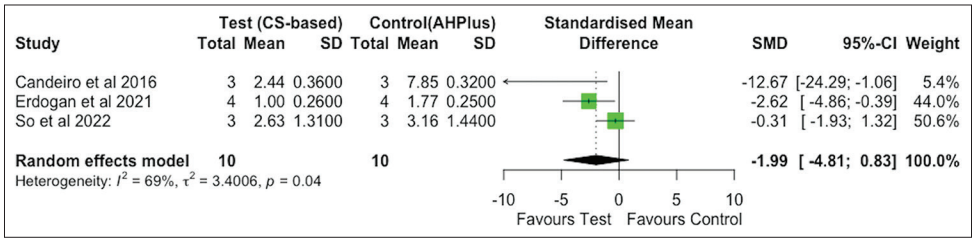

Figure 4: Forest plots of the analysis of genotoxicity as indicated by micronucleus test comparing calcium silicate-based sealer and AH Plus. CI: Confidence interval, SMD: Standardized mean differences, SD: Standard deviation

Root canal sealers can induce an inflammatory response when coming into close contact with periradicular tissues or due to lixiviated components released into surrounding tissues through the apical foramen, dentinal tubules, and accessory or lateral canals.[49,59] The results of the present meta-analysis suggest no difference in the response in the dorsal subcutaneous tissue of rats between premixed calcium silicate-based sealers and AH Plus in an experimental period of 7 days. The inflammatory reaction at the experiment onset occurs due to surgical trauma as well as a reaction to a foreign substance,[6,16,59] which may explain these findings. Moreover, using a calcium silicate-based sealer typically causes an initial inflammatory reaction due to its high alkalinity.[16] Over time, however, the inflammation caused by bioceramic sealers is reduced. The use of AH Plus is associated with significantly greater inflammatory infiltrate in the experimental period of 30 days, suggesting that premixed calcium silicate-based sealers enable the rapid reduction in the initial inflammation and are more biocompatible as the analysis time increases. The basic composition of AH Plus, with the presence of amines to accelerate the polymerization reaction and the release of formaldehyde during the curing process, may explain the greater inflammatory response.[59] The study by Zhang and Peng[59] significantly favored the result in 30 days, as it presents a larger sample size and weight in the meta-analysis.

Cell viability experiments are preliminary biocompatibility tests and constitute the most common assessment of toxicity.[67] This aspect is evident in the present systematic review, as 48 of the 52 studies involved this type of analysis. Cytotoxicity tests define the effect of different materials on cell viability, i.e., they specify the lysis of cells and the inhibition of cell proliferation.[67] Numerous cell viability screening methods are available for studying endodontic materials and the most frequently used is the MTT assay.[2,3,10-12,27,31,32,34,36-38,40,43,46-49,50,52,54,60-62,64,65] This is a colorimetric test that explores the capacity of mitochondrial dehydrogenases (enzymes found only in metabolically viable cells) to cleave the tetrazolium ring, transforming from a compound with a yellow color to one of the dark blue color denominated formazans, which is a crystal that is insoluble in aqueous solutions. Thus, the production of formazan reflects the functional state of the respiratory chain and absorbance depends on the number of living cells.[11]

Adequate contact between test materials and cells is essential in biological analyses and can occur in three forms: direct, indirect, and via extracts.[67] Most studies use extracts for cell viability evaluation,[1,2,4,9,10,12-15,27,29-32,34-40,44-46,48-54,56-58,60-65] whereas few employ indirect contact (sealers on insert)[3,33] or direct contact.[11,28,41,43,47,55] Monolayer cell cultures are satisfactory for cell viability tests of endodontic materials. However, despite being fast, low cost, and informative, cell–extracellular matrix interactions do not form adequately, which diminishes the relevance to clinical situations.[67] Thus, the three-dimensional (3D) cell model has been described to imitate *in vivo* conditions.[67] However, only two studies in the present systematic review used the 3D cell method.[38,47]

Meta-analysis findings suggest that the cell viability of root canal sealers is time and concentration-dependent. AH Plus promoted lower cell viability compared to premixed calcium silicate-based materials in more diluted extracts (1:2 and 1:4) and over time. The studies by Seo *et al*.[11] and Park *et al*.[12] have great weight in the meta-analysis for cell viability at 24 h using undiluted extracts of root canal sealers and cause no significant difference to occur between the materials, since these two studies have the lowest SMDs. However, in the meta-analysis for cell viability at 72 h using undiluted extracts of root canal sealers, studies by Sanz *et al*.,[10] Seo *et al*.,[11] López-García *et al*.,[46] Rodríguez-Lozano *et al*.,[52] and Sanz *et al*.[37] cause a significant difference to occur between the materials, since they have the highest SMDs.

The elemental composition of root canal sealers may also explain the differences in cell viability. The resinous component has been associated with less cell proliferation.[37] The greater cell viability with bioceramic sealers may be related to their alkaline pH, higher release of calcium ions, and formation of hydroxyapatite.[3,54] The divergence among the studies that analyzed cell viability may be explained by the diversity of cells from different sources, as the type of cell can affect the result of the analyses.[67] Stem cells from the human periodontal ligament are the most frequently used,[9,15,37,46,48,52,60,61,64] followed by human gingival fibroblasts,[2,43,45,53,54] fibroblasts from the human periodontal ligament,[4,12,14,28,29] and rat fibroblasts.[34,36,39,63]

Genotoxicity is another critical aspect of biocompatibility that should be considered in choosing a root canal sealer.[54,67] Genotoxicity denotes the presence of a DNA-reactive component that can result in mutagenicity and carcinogenicity.[58] The number of studies that address the genotoxic effect of root canal sealers is low and mainly limited to *in vitro* findings,[64,67] as demonstrated by the inclusion of only five studies involving this type of analysis in the present systematic review.[4,54,58,64,66] Genotoxicity tests are performed to determine the influence of the test material on the genetic material of cells, which can influence cell integrity.[64] The articles included in the quantitative analysis evaluated the genotoxic effects of root canal sealers using the MNT.[4,54,64] The MNT is a reliable method for assessing genotoxicity and is based on the loss of fragments or entire chromosomes during cell mitosis that are not reintegrated to the nucleus, which is therefore transformed into a micronucleus following cell division.[64]

Although the studies by Erdogan *et al*.[4] and Candeiro *et al*.[54] demonstrated that premixed calcium silicate-based sealers have a lower percentage of cells with micronuclei than AH Plus, the study by Só *et al*.[64] had considerable weight in the present meta-analysis. Thus, the quantitative analysis suggests no difference in genotoxic potential as indicated by MNT between bioceramic sealers and AH Plus. As reported for cell viability, the type of cell used for culturing may explain the difference in the results of the studies. While Erdogan *et al*.[4] and Candeiro *et al*.[54] used fibroblasts, Só *et al*.[64] used stem cells. Fibroblasts have a longer useful life in comparison to primary cells. Moreover, the culture of cells from different organs can involve various metabolic enzymes that affect cellular susceptibility.[58]

Calcium silicate-based sealers are expected to have low genotoxicity.[64] A possible explanation for the similar genotoxic effects as indicated by MNT of AH Plus and bioceramic sealers resides in the fact that the resinous compound of the former is diminished when the sealer is diluted, which enables this epoxy resin-based material to have an analogous behavior as that found for calcium silicate-based sealers.[64] Dilution was 1:10 in the studies by Candeiro *et al*.[54] and Só *et al*.[64] and 1:16 in the study by Erdogan *et al*.,[4] which corroborates the previous statement.

SYRCLE RoB[25] is a well-established tool for animal studies. The eligible *in vivo* studies included in the present review had a considerable risk of bias due mainly to the

various methods used and the lack of information on randomization and blinding. However, tools for systematic reviews of *in vitro* studies are still inconsistent. A recent systematic review of *in vitro* studies identified that different quality control tools exist in the literature; however, none covers all necessary critical aspects.[68] In the present study, we used the "Modified CONSORT checklist of items for reporting *in vitro* studies of dental materials,"[26] which has been well accepted in the dental literature.[19] Nevertheless, these guidelines do not provide a quality indicator (high/moderate/low) based on compliance with the proposed items. To overcome this limitation, the percentage of compliance with the items was calculated for each study[19] and varied from 60% to 100% among the studies included in this review, which could be interpreted as a low risk of bias, demonstrating high methodological quality.

Some limitations may be attributed to the present study. First, the methodological heterogeneity of the included studies and lack of standardization in presenting results made it challenging to group data for the quantitative analysis. Thus, 52 studies were included in the systematic review and only 14 in the meta-analysis. Considerable data heterogeneity was detected in the present meta-analysis. Thus, the SMD was used, which is more appropriate for heterogeneous studies,[69] and a sensitivity analysis was performed. Furthermore, the different commercial brands of premixed calcium silicate-based sealers were grouped together, since they belong to the same class of filling materials. However, individual analysis of them could not be performed.

The clinical relevance of data is an important point to be considered in the present review. An agreement was found between *in vivo* and *in vitro* studies, favoring the understanding and enhancing the evidence, as less inflammatory infiltrate and greater cell viability were found with the use of premixed calcium silicate-based sealers compared to AH Plus. However, one should remember that no model can entirely replicate the complex human reactions. Assessments provide only a statistical approximation of biocompatibility; even a root canal sealer classified with high biocompatibility can cause an immediate adverse reaction in individuals.[67] On the other hand, data on the long-term toxicity of root canal sealers are scarce in the dental literature and a material classified initially as an irritant could become biocompatible after 2 or 3 years.[70] It is also important to point out that although root canal sealers can affect treatment success, they cannot be considered the sole cause of endodontic failure. Therapy-related factors (disinfecting procedures such as chemomechanical preparation, irrigating solution, and intracanal medication) and systemic factors (diabetes, hypertension, and menopause/osteoporosis) should also be considered.[71] Finally, calcium silicate-based sealers have excellent biological properties, but AH Plus remains superior in terms of physical–chemical properties, since bioceramic sealers have high solubility and water sorption.[5]

CONCLUSION

The present study found that premixed calcium silicate-based sealers have a better response in the dorsal subcutaneous tissue of rats and lower cytotoxicity compared to the epoxy resin-based sealer AH Plus. In contrast, no significant difference in genotoxicity as indicated by MNT was found in the present systematic review. It is crucial to underscore that the interpretation of results should be approached with caution, given the substantial heterogeneity of data in the current meta-analysis.

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Conflicts of interest
There are no conflicts of interest.

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Supplementary Table 1: Search strategies

Database	Search strategy	Records
PubMed/ MEDLINE	All Fields #1 ((Root Canal Filling Materials[MeSH Terms]) OR (Root Canal Filling Materials)) OR (Root Canal Filling Material)) OR (Root Canal Sealants)) OR (Sealants, Root Canal)) OR (Canal Sealant, Root)) OR (Canal Sealants, Root)) OR (Root Canal Sealant)) OR (Sealant, Root Canal)) OR (Calcium silicate)) OR (Premixed Calcium Silicate-based Sealer)) OR (Premixed Calcium Silicate-based Sealers)) OR (Calcium Silicate- based Sealer)) OR (Calcium Silicate-based Sealers)) OR (Calcium Silicate-Based Root Canal Sealer)) OR (Calcium Silicate-Based Root Canal Sealers)) OR (Calcium Silicate-based Endodontic Sealer)) OR (Calcium Silicate-based Endodontic Sealers)) OR (Calcium Silicate- based Material)) OR (Calcium Silicate-based Materials)) OR (Calcium Silicate–containing Endodontic Sealer)) OR (Calcium Silicate–containing Endodontic Sealers)) OR (Endodontic Sealer based on Calcium Silicate)) OR (Endodontic Sealers based on Calcium Silicates)) OR (Premixed Bioceramic Root Canal Sealer)) OR (Premixed Bioceramic Root Canal Sealers)) OR (Bioceramic Endodontic Sealer)) OR (Bioceramic Endodontic Sealers)) OR (Bioceramic- based Sealer)) OR (Bioceramic-based Sealers)) OR (Bioceramic Root Canal Sealer)) OR (Bioceramic Root Canal Sealers)) OR (Bioceramic-Based Root Canal Sealer)) OR (Bioceramic-Based Root Canal Sealers)) OR (Bioceramic Material)) OR (Bioceramic Materials)) OR (Bioceramic Sealer)) OR (Bioceramic Sealers)) OR (Bioceramic)) OR (Bioceramics) #2 ((((((((((((((((((((((((Epoxy Resins[MeSH Terms]) OR (Root Canal Filling Materials[MeSH Terms])) OR (Epoxy Resins)) OR (Root Canal Filling Materials)) OR (Epoxy Resin)) OR (Resin, Epoxy)) OR (Resins, Epoxy)) OR (Root Canal Filling Material)) OR (Root Canal Sealants)) OR (Sealants, Root Canal)) OR (Canal Sealant, Root)) OR (Canal Sealants, Root)) OR (Root Canal Sealant)) OR (Sealant, Root Canal)) OR (Epoxy Resin-based Root Canal Sealer)) OR (Epoxy Resin-based Root Canal Sealers)) OR (Resin Epoxy–based Endodontic Sealer)) OR (Resin Epoxy–based Endodontic Sealers)) OR (AH Plus)) OR (Conventional Material)) OR (Conventional Materials)) OR (Conventional Resin-Based Sealer)) OR (Conventional Resin-Based Sealers)) OR (Epoxy-based Sealer)) OR (Epoxy-based Sealers)) OR (Epoxy Resin-Based) #3 ((Materials Testing[MeSH Terms]) OR (Subcutaneous Tissue[MeSH Terms])) OR (Cytotoxicity Tests, Immunologic[MeSH Terms])) OR (Cell Culture Techniques[MeSH Terms])) OR (Stem Cells[MeSH Terms])) OR (Mutagenicity Tests[MeSH Terms])) OR (Materials Testing)) OR (Subcutaneous Tissue)) OR (Cytotoxicity Tests, Immunologic)) OR (Cell Culture Techniques)) OR (Stem Cells)) OR (Mutagenicity Tests)) OR (Testing, Materials)) OR (Testing, Biocompatible Materials)) OR (Biocompatible Materials Testing)) OR (Materials Testing, Biocompatible)) OR (Biocompatibility Testing)) OR (Testing, Biocompatibility)) OR (Testings, Biocompatibility)) OR (Subcutaneous Tissues)) OR (Tissue, Subcutaneous)) OR (Tissues, Subcutaneous)) OR (Cytotoxicity Tests, Immunological)) OR (Cytotoxicity Test, Immunological)) OR (Immunological Cytotoxicity Test)) OR (Immunological Cytotoxicity Tests)) OR (Immunologic Cytotoxicity Tests)) OR (Cytotoxicity Test, Immunologic)) OR (Immunologic Cytotoxicity Test)) OR (MTT)) OR (MTT test)) OR (Cell Culture Technique)) OR (Culture Technique, Cell)) OR (Culture Techniques, Cell)) OR (Cell Culture)) OR (Cell Cultures)) OR (Cell, Stem)) OR (Cells, Stem)) OR (Stem Cell)) OR (Mutagenicity Test)) OR (Toxicity Tests, Genetic)) OR (Mutagen Screening)) OR (Mutagen Screenings)) OR (Screening, Mutagen)) OR (Screenings, Mutagen)) OR (Tests, Genetic Toxicity)) OR (Genetic Toxicity Test)) OR (Toxicity Test, Genetic)) OR (Genetic Toxicity Tests)) OR (Genotoxicity Tests)) OR (Genotoxicity Test)) OR (Test, Genotoxicity)) OR (Tests, Genotoxicity)) OR (Biocompatibility)) OR (Tissue response)) OR (Cytotoxicity)) OR (Cytocompatibility)) OR (Genotoxicity)) OR (Biological Property)) OR (Biological Properties)) OR (Biological Effect)) OR (Biological Effects)) OR (Biological Response)) OR (Biological Responses) ((#1) AND (#2)) AND (#3)	*n*=3852
Embase	(''calcium silicate''/exp OR ''calcium kaolinite'' OR ''calcium silicate'' OR ''kaolinite calcium'' OR ''pseudowollastonite'' OR ''silicate calcium'' OR ''wollastonite'' OR ''bioceramics''/exp OR ''bioceramics'' OR ''root canal filling material''/exp OR ''endorez'' OR ''proroot mta'' OR ''dental root filling material'' OR ''root canal filling material'' OR ''root canal filling materials'' OR ''root canal sealant'' OR ''root canal sealer'' OR ''root filling material'' OR ''tooth root canal sealing agent'' OR ''premixed calcium silicate-based sealer'' OR ''premixed calcium silicate-based sealers'' OR ''calcium silicate-based sealer'' OR ''calcium silicate-based sealers'' OR ''calcium silicate-based root canal sealer'' OR ''calcium silicate-based root canal sealers'' OR ''calcium silicate-based endodontic sealer'' OR ''calcium silicate-based endodontic sealers'' OR ''calcium silicate-based material'' OR ''calcium silicate-based materials'' OR ''calcium silicate–containing endodontic sealer'' OR ''calcium silicate–containing endodontic sealers'' OR ''endodontic sealer based on calcium silicate'' OR ''endodontic sealers based on calcium silicates'' OR ''premixed bioceramic root canal sealer'' OR ''premixed bioceramic root canal sealers'' OR ''bioceramic endodontic sealer'' OR ''bioceramic endodontic sealers'' OR ''bioceramic-based sealer'' OR ''bioceramic-based sealers'' OR ''bioceramic root canal sealer'' OR ''bioceramic root canal sealers'' OR ''bioceramic-based root canal sealer'' OR ''bioceramic-based root canal sealers'' OR ''bioceramic material'' OR ''bioceramic materials'' OR ''bioceramic sealer'' OR ''bioceramic sealers'') AND (''epoxy resin''/exp OR ''epoxide resin'' OR ''epoxy resin'' OR ''epoxy resins'' OR ''epoxyresin'' OR ''resin cement''/exp OR ''ah plus'' OR ''acroseal'' OR ''adper single bond 2'' OR ''excite (drug)'' OR ''filtek supreme ultra'' OR ''filtek z250'' OR ''maxcem elite'' OR ''meliodent'' OR ''panavia f2.0'' OR ''realseal'' OR ''renamel'' OR ''paladur'' OR ''resin cement'' OR ''resin cements'' OR ''self-cured dental restorative composite resin'' OR ''root canal filling material''/exp OR ''endorez'' OR ''proroot mta'' OR ''dental root filling material'' OR ''root canal filling material'' OR ''root canal filling materials'' OR ''root canal sealant'' OR ''root canal sealer'' OR ''root filling material'' OR ''tooth root canal sealing agent'' OR ''epoxy resin based root canal sealer''/exp OR ''epoxy resin-based root canal sealers'' OR ''resin epoxy–based endodontic sealer'' OR ''resin epoxy–based endodontic sealers'' OR ''conventional material'' OR ''conventional materials'' OR ''conventional resin-based sealer'' OR ''conventional resin-based sealers'' OR ''epoxy-based sealer'' OR ''epoxy-based sealers'' OR ''epoxy resin-based'') AND (''biocompatibility''/exp OR ''biocompatibility'' OR ''cytotoxicity''/exp OR ''cell toxicity'' OR ''cytotoxic activity'' OR ''cytotoxic effect'' OR ''cytotoxic reaction'' OR ''cytotoxicity'' OR	*n*=2060

Contd...

Supplementary Table 1: Contd...

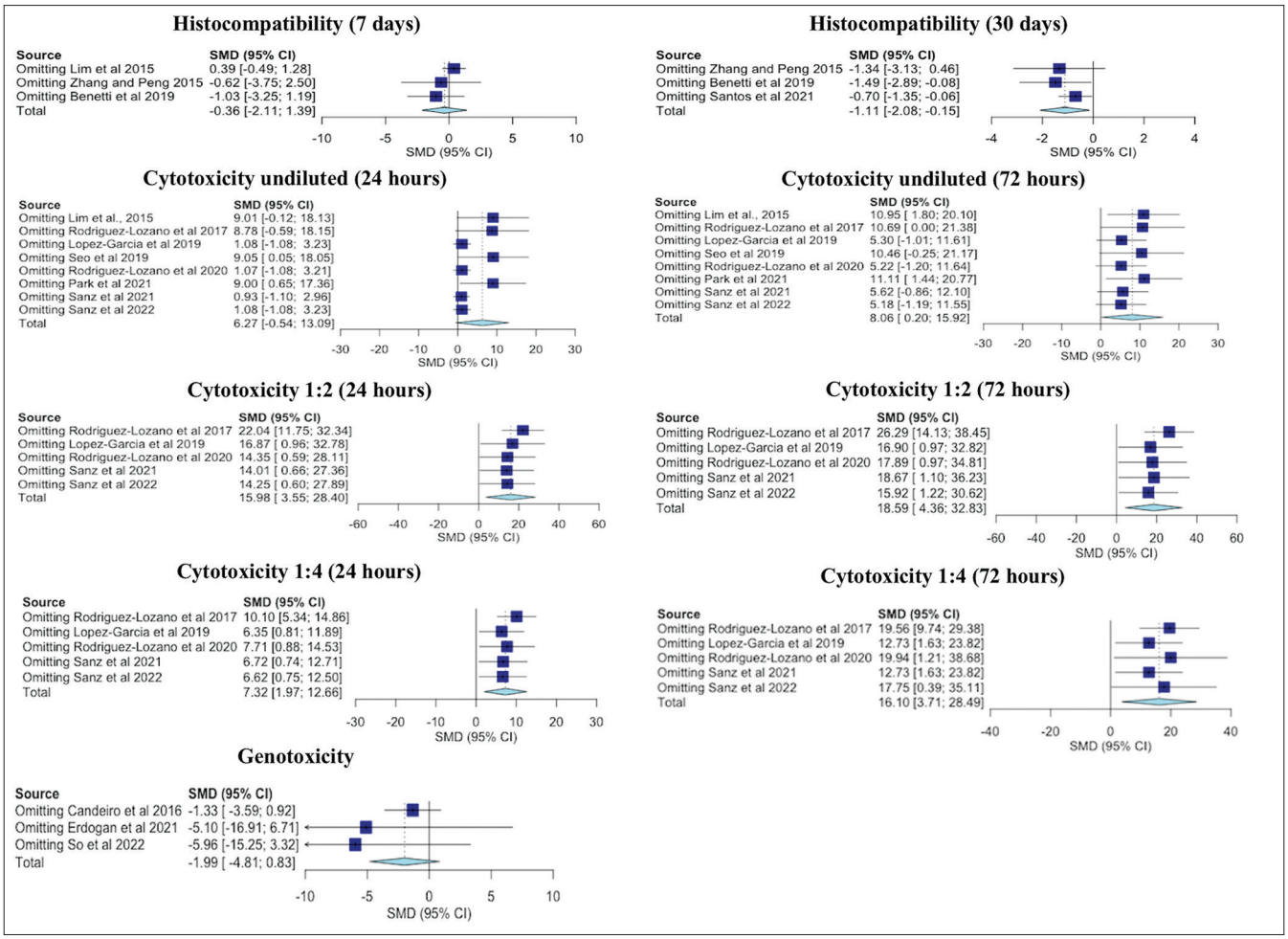
Database	Search strategy	Records
	"cytotoxicity assay"/exp OR "cell toxicity assay" OR "cytotoxicity assay" OR "mtt assay"/exp OR "3- (4, 5-dimethylthiazol-2-yl) -2, 5- diphenyltetrazolium bromide assay" OR "mtt assay" OR "cell culture technique"/exp OR "cell culture method" OR "cell culture technique" OR "cell culture techniques" OR "culture technique" OR "culture techniques" OR "genotoxicity"/exp OR "genotoxicity" OR "genotoxicity assay"/exp OR "genotoxicity assay" OR "subcutaneous tissue"/exp OR "hypodermis" OR "subcutaneous tissue" OR "subcutis" OR "tela subcutanea" OR "stem cell"/exp OR "cell, stem" OR "precursor cell" OR "progenitor cell" OR "stem cell" OR "stem cells" OR "mutagen testing"/exp OR "mutagen screening" OR "mutagen testing" OR "mutagenicity test" OR "mutagenicity tests" OR "testing, mutagen" OR "materials testing"/exp OR "material testing" OR "materials testing" OR "testing, material" OR "tissue response" OR "cytocompatibility"/exp OR "biological property" OR "biological properties" OR "biological effect" OR "biological effects" OR "biological response"/exp OR "biological responses")	
Google Scholar	(root canal filling materials OR root canal sealants OR premixed calcium silicate-based sealer OR bioceramic endodontic sealer) AND (epoxy resins OR AH Plus) AND (materials testing OR subcutaneous tissue OR cytotoxicity tests OR cell culture techniques OR stem cells OR mutagenicity tests OR biocompatibility OR MTT OR genotoxicity OR biological properties)	*n*=332
OpenGrey Europe*	(root canal filling materials OR root canal sealants OR premixed calcium silicate-based sealer OR bioceramic endodontic sealer) AND (epoxy resins OR AH Plus) AND (materials testing OR subcutaneous tissue OR cytotoxicity tests OR cell culture techniques OR stem cells OR mutagenicity tests OR biocompatibility OR MTT OR genotoxicity OR biological properties)	*n*=64
ProQuest	Academic Journals (root canal filling materials OR root canal sealants OR premixed calcium silicate-based sealer OR bioceramic endodontic sealer) AND (epoxy resins OR AH Plus) AND (materials testing OR subcutaneous tissue OR cytotoxicity tests OR cell culture techniques OR stem cells OR mutagenicity tests OR biocompatibility OR MTT OR genotoxicity OR biological properties)	*n*=1417

*GreyNet has recently archived OpenGrey in its collection of research data housed in the Data Archiving and Networked Services (DANS) EASY Archive (https://easy.dans.knaw.nl/ui/home)

Supplementary Table 2: : Justification for not including each study in the meta-analysis

Property	Study	Reason for not including meta-analysis
Histocompatibility	Alves Silva *et al*., 2020[6]	Results presented as numerical density of inflammatory cells/mm^2
	Ferreira *et al*., 2022[13]	Different classification of inflammatory infiltrate scores
Cytotoxicity	Zhang *et al*., 2010[50]	Cell viability was calculated as the percentage relative to the control group
	Zhang *et al*., 2010[34]	Cell viability was calculated as the percentage relative to the control group
	Loushine *et al*., 2011[3]	Cell viability was calculated as the percentage relative to the control group
	Willershausen *et al*., 2011[28]	Alamar Blue assay and ToxiLight BioAssay Kit
	Zoufan *et al*., 2011[36]	Cell viability was calculated as the percentage relative to the control group
	Güven *et al*., 2013[33]	MTS assay
	Kim and Shin, 2014[45]	WST-1 assay
	Zhou *et al*., 2015[53]	Quantitative flow cytometry
	Candeiro *et al*., 2016[54]	Different dilution of extracts
	Eldeniz *et al*., 2016[58]	XTT assay
	Silva *et al*., 2016[38]	Cell viability was calculated as the percentage relative to the control group
	Da Silva *et al*., 2017[47]	3D cell culture
	Alsubait *et al*., 2018[1]	Alamar Blue assay
	Kebudi Benezra *et al*., 2018[43]	Different dilution of extracts
	Beshr and Abdelrahim, 2018[32]	Different dilution of extracts
	Colombo *et al*., 2018[2]	Cell viability was calculated as the percentage relative to the control group
	Taraslia *et al*., 2018[55]	Costar Transwell assay
	Benetti *et al*., 2019[39]	Alamar Blue assay
	Giacomino *et al*., 2019[51]	Luminescence assay based on adenosine triphosphate quantification
	Lee *et al*., 2019[61]	Cell viability was calculated as the percentage relative to the control group
	Lee *et al*., 2019[56]	WST-1 assay
	Mestieri *et al*., 2019[62]	Cell viability was calculated as the percentage relative to the control group
	Souza *et al*., 2019[41]	Annexin-V/Propidium Iodide double stain using the FACSCalibur cytometer
	Zordan-Bronzel *et al*., 2019[31]	Cell viability was calculated as the percentage relative to the control group
	Almeida *et al*., 2020[40]	Different dilution of extracts
	Jo *et al*., 2020[9]	kit-8 (CCK-8)
	Oh *et al*., 2020[15]	kit-8 (CCK-8)
	Zheng *et al*., 2020[48]	Cell viability was calculated as the percentage relative to the control group
	Erdogan *et al*., 2021[4]	XTT assay
	Jun *et al*., 2021[44]	WST-8 assay
	Saghiri *et al*., 2021[63]	MTS assay
	Zordan-Bronzel *et al*., 2021[35]	Cell viability was calculated as the percentage relative to the control group
	Ferreira *et al*., 2022[13]	Different dilution of extracts
	Janini *et al*., 2022[27]	Unsuccessful contact – no standard deviation
	Mann *et al*., 2022[14]	XTT assay
	Sheela *et al*., 2022[57]	XTT assay
	Só *et al*., 2022[64]	Different dilution of extracts
	Wuersching *et al*., 2022[29]	WST-8 assay
	Martorano *et al*., 2023[65]	Different dilution of extracts
	Souza *et al*., 2023[30]	XTT assay
Genotoxicity	Eldeniz *et al*., 2016[58]	c-H2AX focus assay
	Siregar *et al*., 2018[66]	γ-H2AX assay

MTS: (3-(4, 5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy- phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium), WST: Water-soluble tetrazolium salt, XTT: (sodium 30-[1- (phenyla minocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate), 3D: Three dimension



Supplementary Figure 1: Sensitivity analyses