



# Cytocompatibility of Three Calcium Silicate-Based Biomaterials: A Systematic Review and Meta-Analysis

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## Abstract

**Introduction:** To systematically evaluate and compare the *in vitro* cytotoxic effects of three calcium silicate-based cements [Mineral Trioxide Aggregate (MTA), Biodentine, and Calcium Enriched Mixture (CEM) Cement], focusing on their biocompatibility with human dental pulp cells in vital pulp therapy (VPT). **Materials and Methods:** A comprehensive search was conducted in PubMed, Scopus, Web of Science, Cochrane, ProQuest, Wiley, and Ovid (including Embase) for *in vitro* studies published up to February 2023. Studies evaluating cytotoxicity via cell viability assays (e.g., MTT) were included. **Results:** Among 8,004 screened records, 32 studies met the inclusion criteria, with 11 included in the meta-analysis. Overall, no significant differences in cytotoxicity were identified among Mineral Trioxide Aggregate (MTA), Biodentine, and Calcium-Enriched Mixture (CEM) cement. Nevertheless, undiluted MTA and Biodentine at a 1:4 dilution were associated with a statistically significant reduction in cell viability at 48 hours ( $P < 0.05$ ). No other concentrations or exposure durations showed significant cytotoxic effects. CEM cement consistently demonstrated favorable biocompatibility across the included studies. **Conclusion:** These findings indicate that MTA, Biodentine, and CEM cement generally exhibit acceptable *in vitro* biocompatibility for vital pulp therapy. Their biological responses may be influenced by material concentration and exposure duration, emphasizing the importance of careful and controlled clinical application.

**Keywords:** Biocompatibility; Cytotoxicity; MTA; Biodentine; CEM Cement; Vital Pulp Therapy

## Introduction

Endodontic therapy necessitates the eradication of infection from the root canal system and its subsequent obturation in a three-dimensional manner [1]. The final stage of this process involves the intricacies of filling the root canal system with a variety of techniques at one's disposal. The quintessential method employs semi-solid entities, such as gutta-percha, accompanied by an adhesive sealant [2]. Root canal filling material must exhibit characteristics such as adherence to dentine, hermetic sealing capabilities, non-toxicity, dimensional fidelity, and minimal solubility [3]. These materials must be

biologically compatible and withstand proximity to periradicular tissues over prolonged periods without eliciting cytotoxic or mutagenic reactions, which may precipitate inflammation or neoplastic cellular transformations [4].

A plethora of materials have been trialed for root-end filling, including, but not limited to, gutta-percha, zinc oxide eugenol, intermediate restorative materials (IRMs) and glass ionomer. However, none possess the comprehensive suite of ideal attributes [5, 6]

Recent advances have led to the emergence of bioactive and bio-regenerative materials in endodontics, marking a paradigm shift in root canal therapies [1]. The emergence and subsequent refinement of calcium silicate-based cements have announced a

new era in restorative dentistry and endodontic tissue engineering. Such materials have become a cornerstone in the realm of regenerative endodontic procedures. Notably, this category includes essential compounds such as mineral trioxide aggregate (MTA), Biodentine (BD), and calcium-enriched mixture (CEM) cement, each playing a pivotal role in the advancement of endodontic therapeutics [2, 7].

Mineral trioxide aggregate (MTA), a biomaterial widely used in endodontic treatments, was first introduced by Mahmoud Torabinejad at Loma Linda University in the USA in 1993. Exhibiting exceptional biocompatibility, MTA is formulated as a hydrophilic/Portland cement-based powder that demonstrates excellent clinical performance, particularly in the presence of moisture [3, 8]. Its distinctive properties, which include the ability to remain stable in fluid environments, possess adequate radiopacity, maintain a high pH level, resist expansion, and demonstrate low solubility, have rendered it the material of choice for a wide range of endodontic treatments [9]. These treatments encompass the regeneration of vital pulp, the remediation of perforations within the root structure, apexification, and the obturation of the root-end cavities [4, 10, 11]. The specific mechanisms by which MTA facilitates the differentiation of human dental pulp stem cells (hDPSC) into odontoblasts remain to be conclusively determined. However, the role of calcium ions is presumed to be significant in this biological process [5].

Biodentine, a novel constituent of the Portland cement lineage, exhibits mechanical equivalence to dentine and has been engineered to enhance its physicochemical and biological characteristics. In its powdered manifestation, Biodentine comprises tricalcium silicate, dicalcium silicate, calcium carbonate, and zirconium oxide, which serve to impart opacity. When liquid, it incorporates calcium chloride within an aqueous medium, synergistically blended with polycarboxylate to function as a dispersing agent [6].

Calcium-enriched mixture (CEM) cement, introduced into the dental scientific arena in 2006, is a composite material comprised of a diverse array of calcium-based compounds, including calcium oxide, calcium hydroxide, calcium carbonate, calcium silicate, and calcium phosphate. This admixture boasts extensive clinical applications within the endodontic field, including various vital pulp therapies/techniques, regenerative procedures/revascularization, managing various internal and external root resorptions, apical plug/intra-orifice barriers, and repair of perforations [12-25].

Despite sharing a broad spectrum of clinical applications with MTA, CEM cement distinguishes itself through its unique chemical formulation, which diverges from both MTA and traditional Portland cement [26]. CEM cement exhibits superior physical attributes, notably in its rheological properties and film

thickness, alongside a more rapid setting time when compared to MTA [12]. Moreover, it demonstrates a markedly enhanced sealing capacity relative to IRMs and presents a marginally reduced incidence of microleakage compared to MTA [27]. Regarding cytotoxicity, the effects of CEM cement in cellular culture assays align closely with those of MTA [28, 29]. Furthermore, its compatibility with vital pulp therapy is comparable to that of MTA, with a noted advantage over the use of calcium hydroxide [30]. These three specific cases are introduced as leading hydraulic calcium silicate cements (CSCs) for clinical practice [31, 32].

The literature confirms that while CSCs generally exhibit superior biocompatibility, clinical protocol critically influences their biological success [33]. Specifically, the biological success of CSCs varies concerning their regenerative potential: CEM cement may promote higher proliferation (growth) of human dental pulp stem cells (hDPSCs), while Biodentine significantly induces a greater secretion of Transforming Growth Factor-beta 1 (TGF- $\beta$ 1), a key differentiation factor, suggesting distinct molecular pathways for dentinogenesis [34]. Also, comparative studies on Stem Cells from the Apical Papilla (SCAPs) show that while contemporary CSCs are cytocompatible, Biodentine often exhibits a superior capacity for stimulating alkaline phosphatase (ALP) activity and subsequent mineralization [35, 36].

Successful vital pulp therapy hinges on various critical factors: a healthy host (good healing capacity), a sterile pulp environment, and the use of biocompatible materials [37, 38]. Biocompatibility is a broad concept requiring rigorous evaluation through both *in vitro* and *in vivo* methods [39]. Recognizing that animal models can display different metabolic and immunological responses than human tissues [33], this systematic review and meta-analysis represents the first comprehensive evaluation of its kind. It focuses on the cytotoxic effects of these biomaterials on human cell lines, examining variables such as exposure duration, concentration, and cell lineage specificity.

## Materials and Methods

### Study Registration

The protocol for this systematic review was registered with PROSPERO (International Prospective Register of Systematic Reviews) under registration number CRD42024500255. The full protocol can be accessed at: <https://www.crd.york.ac.uk/PROSPERO/view/CRD42024500255>

### Search Strategy

This systematic review adhered to the protocols delineated by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. (<http://www.prisma-statement.org>).

A comprehensive literature search was executed on February 16, 2023, across multiple databases, including PubMed, Web of Science, Scopus, Embase, Cochrane, ProQuest, and Wiley. The search strategy was meticulously performed in a tripartite procedure following a PICO analysis to identify the relevant search terms pertinent to the research query. This strategy involved integrating synonyms, acronyms, variant spellings across UK/US English, singular/plural word forms, and thesaurus terms to ensure thorough conceptual retrieval. Using thesaurus tools, MeSH, and Emtree facilitated the refinement of keywords for thematic database searches where applicable. A preliminary search, supplemented by examining keywords from seminal articles in the domain, was instrumental in augmenting and refining the search lexicon. The foundational search syntax employed was: (MTA OR "Mineral trioxide aggregate" OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) AND (Toxic\* OR Cytotoxicit\*). Detailed search strategies for each database are delineated in Appendix 1. Additionally, a manual search of the references from all included studies and pertinent systematic reviews was undertaken to identify any potentially qualifying studies, supplemented by citation tracking of included articles. [Table 1](#) presents the search strategy used in the databases.

### PICO Question

The framework for this structured inquiry was predicated on the PICO strategy (Population, Intervention, Comparison, and Outcome) as outlined in the PRISMA-P 2016:

- Population (P): All articles encompassing a minimum of two biomaterials.
- Intervention (I): Examining the cytotoxicity of MTA, Biodentine, and CEM cement.
- Comparison (C): Assessment of the cytotoxicity among the three materials (MTA, Biodentine, and CEM cement).
- Outcome (O): Ascertainment of the relative toxicity of the biomaterials under study.

### Data Compilation and Quality Assessment

Two researchers (A. R. and M. M.) independently extracted data, with any discrepancies resolved through discussion. If consensus remained elusive, a third researcher (E.K) arbitrated. The data procured included article title, primary author, publication year, sample size, study design, cell culture, biomaterial type, cell type, concentration, cell exposure time, and mean values, as well as cytotoxicity's standard errors or confidence intervals. The eligibility of studies was independently assessed by two authors, with full-text reviews conducted on eligible studies. Any discrepancies were resolved through dialogue or third-party intervention. The ESA protocol

appraisal tool was employed for the quality assessment of the included studies. The ESA Quality Assessment Checklist for In-Vitro Studies is a structured tool developed by expert to evaluate the quality of in-vitro experiments. It evaluates three core domains: reporting quality, replicability, and internal/external validity. The checklist was developed in consultation with experts and adapted from several validated tools and literature sources [40, 41]. The checklist was uploaded in the attachments section. The study selection process is summarized in [Table 2](#).

### Data analysis

All statistical analyses, including the meta-analysis, were performed using Stata software (version 16). Descriptive statistics were used to summarize the baseline characteristics of the included studies. In studies reporting optical density (OD) values (e.g., MTT or MTS assays), OD was not considered an independent outcome. Instead, all OD data were converted into normalized cell viability percentages using the formula reported in the original studies ( $\text{cell viability (\%)} = [\text{OD of experimental group} / \text{OD of control group}] \times 100$ ) prior to data extraction. Meta-analyses were conducted exclusively using cell viability as the unified outcome measure [42].

Given the anticipated methodological and clinical heterogeneity among studies, a random-effects model was applied to estimate pooled effect sizes. Effect sizes were expressed as standardized mean differences (SMDs) with corresponding 95% confidence intervals (CIs), allowing comparison across studies employing different experimental conditions and measurement scales. Statistical heterogeneity was assessed using the  $I^2$  statistic [42]. When substantial heterogeneity was detected, subgroup analyses were performed to explore potential sources of variability, including substance concentration and cell exposure duration. All statistical tests were two-tailed, and a  $P$ -value of  $<0.05$  was considered statistically significant.

## Results

### Search Results and Study Characteristics

Based on the search in databases using the described search strategy, 8,004 article titles were initially found. After entering all articles into EndNote 8 software, 2809 duplicate article titles were removed. Among the remaining 5,195 article titles, 4,666 articles were removed due to unrelated titles, and as a result, 529 articles remained. Of these remaining articles, 497 were excluded based on the inclusion and exclusion criteria. After reviewing the full text of the articles, 32 studies that met the screening criteria were approved for systematic review, and 11 studies were included in the meta-analysis ([Fig. 1](#)).

**Table 1.** Search strategy and terms used to identify studies

Database	Formula	Results
PubMed	((("Mineral trioxide aggregate*" [Title/Abstract] OR "MTA cement" [Title/Abstract] OR MT aggregate[Title/Abstract] OR aggregate ProRoot[Title/Abstract] OR OrthoMTA[Title/Abstract] OR RetroMTA[Title/Abstract] OR MTA-Fillapex[Title/Abstract] OR Biodentine[Title/Abstract] OR "CEM Cement" [Title/Abstract] OR "Calcium enriched mixture" [Title/Abstract] OR "Calcium silicate based cement" [Title/Abstract] OR CSC[Title/Abstract]) AND (Toxic*[Title/Abstract] OR Cytotoxicit*[Title/Abstract])) OR ((MTA[Title/Abstract] OR "Mineral trioxide aggregate" [Title/Abstract] OR Biodentine[Title/Abstract] OR "CEM Cement" [Title/Abstract] OR "Calcium enriched mixture" [Title/Abstract] OR "Calcium silicate based cement" [Title/Abstract] OR CSC[Title/Abstract]) AND (("Toxic Actions" [Mesh]) OR "toxicity" [Subheading])) OR ((( "calcium-enriched mixture cement" [Supplementary Concept]) OR ("mineral trioxide aggregate" [Supplementary Concept])) AND (("Toxic Actions" [Mesh]) OR "toxicity" [Subheading]))	1928
Web of Science	Toxic* OR Cytotoxicit* (Topic) and "Mineral trioxide aggregate*" OR "MTA cement" OR "MT aggregate" OR aggregate ProRoot OR OrthoMTA OR RetroMTA OR MTA-Fillapex OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC (Topic)	1181
Scopus	( TITLE-ABS-KEY ("mineral trioxide aggregate*" OR "mta cement" OR "mt aggregate" OR aggregate AND proroot OR orthomta OR retromta OR mta-fillapex OR Biodentine OR "cem cement" OR "calcium enriched mixture" OR "calcium silicate based cement" OR csc ) AND TITLE-ABS-KEY ( toxic* OR cytotoxicit* ) )	1641
Cochrane	(MTA OR "Mineral trioxide aggregate" OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) AND (Toxic* OR Cytotoxicit*) in Title Abstract Keyword - (Word variations have been searched)	163 (1 Review, 162 Trials)
ProQuest	(abstract("Mineral trioxide aggregate*" OR "MTA cement" OR "MT aggregate" OR aggregate ProRoot OR OrthoMTA OR RetroMTA OR MTA-Fillapex OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) OR mainsubject("Mineral trioxide aggregate*" OR "MTA cement" OR "MT aggregate" OR aggregate ProRoot OR OrthoMTA OR RetroMTA OR MTA-Fillapex OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) OR title("Mineral trioxide aggregate*" OR "MTA cement" OR "MT aggregate" OR aggregate ProRoot OR OrthoMTA OR RetroMTA OR MTA-Fillapex OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC)) AND (abstract(Toxic* OR Cytotoxicit*) OR mainsubject(Toxic* OR Cytotoxicit*) OR title(Toxic* OR Cytotoxicit*))	305
Wiley	("MTA OR "Mineral trioxide aggregate" OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) AND (Toxic* OR Cytotoxicit*) in Abstract	206
Ovid (All Database included Embase)	((Mineral trioxide aggregate or MTA cement or MT aggregate or aggregate ProRoot or OrthoMTA or RetroMTA or MTA-Fillapex or Biodentine or CEM Cement or Calcium enriched mixture or Calcium silicate based cement or CSC).ab. or (Mineral trioxide aggregate or MTA cement or MT aggregate or aggregate ProRoot or OrthoMTA or RetroMTA or MTA-Fillapex or Biodentine or CEM Cement or Calcium enriched mixture or Calcium silicate based cement or CSC).ti. or (Mineral trioxide aggregate or MTA cement or MT aggregate or aggregate ProRoot or OrthoMTA or RetroMTA or MTA-Fillapex or Biodentine or CEM Cement or Calcium enriched mixture or Calcium silicate based cement or CSC).kf.) AND ((Toxic* or Cytotoxicit*).ab. or (Toxic* or Cytotoxicit*).ti. or (Toxic* or Cytotoxicit*).kf.))	2580
<b>Total= 8004; After deleting duplicated files= 5195</b>		

Main Syntax: (MTA OR "Mineral trioxide aggregate" OR Biodentine OR "CEM Cement" OR "Calcium enriched mixture" OR "Calcium silicate based cement" OR CSC) AND (Toxic\* OR Cytotoxicit\*)

**Table 2.** Entry and exit criteria for studies

Characteristics	Inclusion Criteria	Exclusion Criteria
Publication year	Studies published from the beginning to February 16, 2023	Studies published after this time
Language	English	Other languages other than English
Population	Human cell lines	Other types of cell lines, Such as animal Cell Lines
Materials tested	At least two different materials compared with each other	Materials compared only to the control

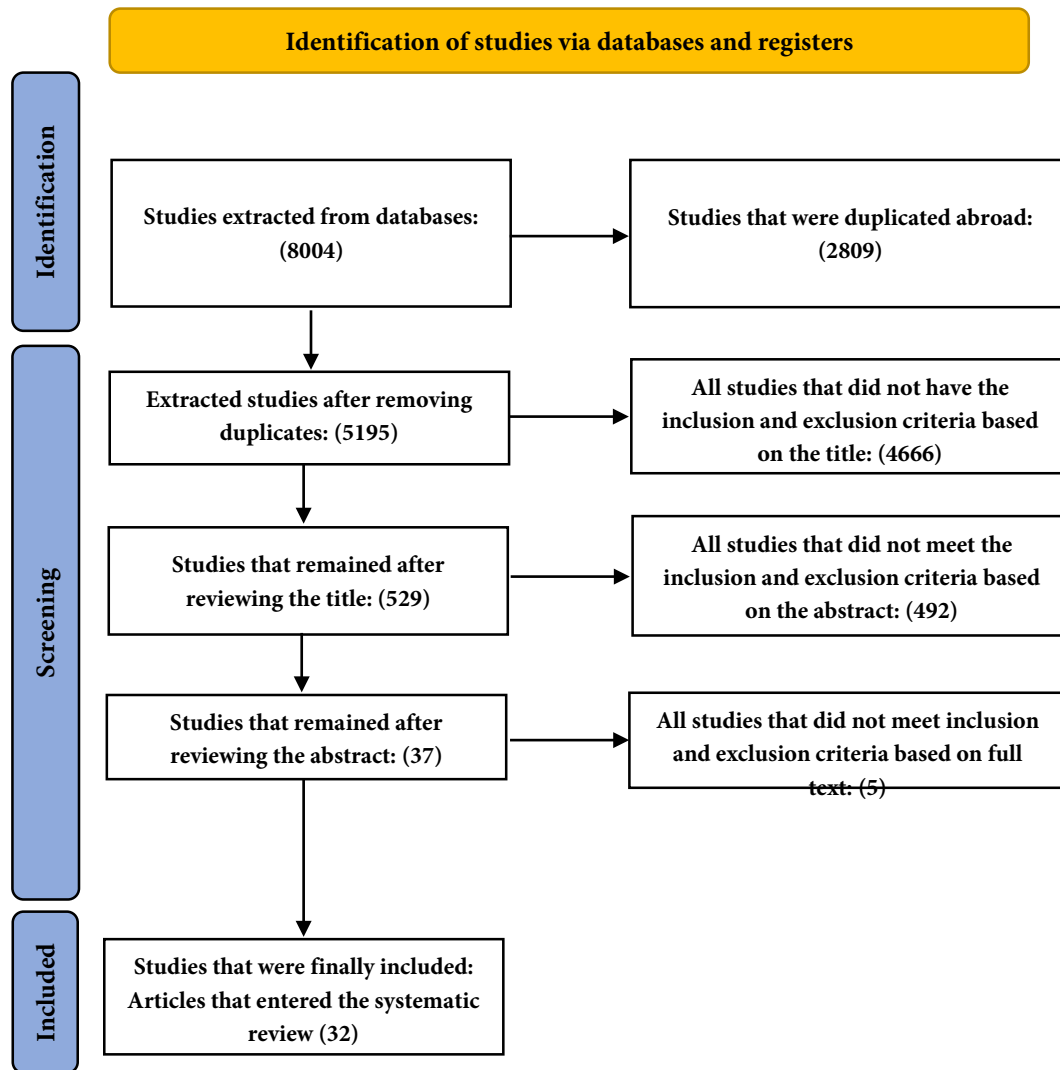


Figure 1. PRISMA flowchart for reviewing systematic review studies

### Quality assessment

Each article's data was extracted separately (Table 3 in the appendices), and each quality was scored based on the ESA Protocol checklist (Table 4). A score of 6 or higher was considered fair quality; otherwise, it was considered low quality. The ESA Protocol checklist is included in the appendices section for further reading.

### Characteristics of studies included in the meta-analysis

The eleven studies incorporated into the quantitative synthesis (meta-analysis) are summarized in Table 5. These studies evaluated the cytocompatibility of Mineral Trioxide Aggregate (MTA), Biodentine, and/or Calcium-Enriched Mixture (CEM) cement on various human cell lineages. The most frequently tested cell types were human dental pulp stem cells (hDPSCs/DPSCs, 6 studies), followed by human gingival fibroblasts (HGFs, 2 studies), stem cells from human exfoliated deciduous teeth (SHED/SHEDs, 2

studies), and human bone marrow-derived mesenchymal stem cells (hBMSCs, 2 studies). The concentration of material extracts varied across studies, with undiluted (non-diluted) extracts being the most common, followed by serial dilutions of 1:2 and 1:4. Cell exposure times ranged from 24 hours to 14 days, with 24, 48, and 72 hours being the most frequently assessed intervals. The primary method for assessing cytotoxicity was the MTT assay (9 studies), while one study utilized flow cytometry and another employed the MTS assay. The heterogeneity in experimental parameters was accounted for by employing a random-effects model in the meta-analysis.

### Meta-analysis:

Only eleven studies related to the investigation of the cytotoxicity of MTA, Biodentine, and CEM Cement in different cell lines were included in the meta-analysis.

**Table 3.** Review of studies conducted on MTA, Biodentine and Cem cement

Row	Names of authors	Year	Type of calcium silicate base cement (MTA OR Biodentine OR Cem cement)	Type of cell line used	Substance concentration	Cell exposure time	Measurement method	Results
1	Khedmat et al	2013	Biodentine (Septodont), Calcium Enriched Mixture (CEM cement, BioniqueDent), and ProRoot MTA (Dentsply Tulsa Dental).	Human monocytes were isolated from peripheral blood mononuclear cells	-	24 hr 7 day 28day	MTT assay	Biodentine and ProRoot MTA had similar biocompatibility. Biosealer and CEM cement after 48 hr of incubation were significantly more cytotoxic.
2	Omidi et al	2019	Angelus mineral trioxide aggregate (MTA; Angelus), calcium-enriched mixture (CEM; BioniqueDent), and Biodentine (Septodont)	human dental pulp stem cells (hDPSCs)	Non diluted 1:2 1:4 1:8 1:16 1:32	24 hr 48 hr 72 hr	MTT assay	The biomaterials of MTA, CEM, and Biodentine at different dilutions had no cytotoxic effects on hDPSCs at different time.
3	Saberi et al	2015	mineral trioxide aggregate (MTA), calcium-enriched mixture (CEM) cement, Biodentine (BD)	stem cells of the human apical papilla (SCAP)	1 mg of each biomaterial	24 hr 48 hr 168 hr	MTT assay	Cytotoxicity of the four materials was not significantly different from that of the control group at 24, 48 and 168 h ( $P>0.05$ ).
4	Saberi et al	2016	mineral trioxide aggregate (MTA), calcium enriched mixture (CEM) cement, Biodentine (BD)	human gingival fibroblasts (HGFs)	1 mg of each biomaterial	24 hr 48 hr 168 hr	MTT assay	Cytotoxicity of MTA, CEM, Biodentine and OCP against HGFs was similar to that of the control group at 24 and 48 hours. Over time, MTA and Biodentine exhibited less cytotoxicity than other materials.
5	Küçükkaya et al	2016	MTA, calcium enriched mixture (CEM) cement, and Biodentine	human periodontal ligament fibroblasts	-	24 hr 48 hr 72 hr	WST-1 assay	There was no significant difference in cell viability amongst the test materials after each incubation period ( $P>0.05$ ).
6	Zhou et al	2013	Biodentine, White Pro Root mineral trioxide aggregate (MTA)	human gingival fibroblasts	1:1 1:2 1:4 1:8	1day 3 day 7 day	flow cytometry	Cells exposed to extracts from Biodentine and MTA showed the highest viabilities at all extract concentrations. ( $P < .05$ ). There was no significant difference in cell viabilities between Biodentine and MTA during the entire experimental period ( $P>0.05$ ).
7	Youssef et al	2019	mineral trioxide aggregate (MTA), Biodentine	dental pulp stem cells (DPSCs)	0.5 mg/ml	3 day	MTT assay	All capping materials showed variable cytotoxicity against DPSCs (77% for Emdogain, 53% for MTA, 26% for Biodentine and 16% for Ca(OH) <sub>2</sub> compared to control ( $P$ value $< 0.0001$ ). Our results suggest MTA, Biodentine and Emdogain exhibit similar attributes and may score better than Ca(OH) <sub>2</sub> .
8	Weekate et al	2021	Biodentine, ProRoot MTA	lipopolysaccharide (LPS)-induced human dental pulp cells (hDPCs)	-	24 hr 48 hr	Alamar blue assay	Both Biodentine and MTA extracts promoted cell survival, whereas the combination of NAC to these material extracts significantly increased the number of viable cells at 24 h ( $P<0.05$ ).

9	Tomas-Catala et al	2018	MTA Repair HP, NeoMTA Plus, and Biodentine	human dental pulp stem cells (hDPSCs)	Non diluted 1:2 1:4	24 hr 48 hr 72 hr	MTT assay	Cell viability was moderate after 24 and 48 hours in the presence of MTA Repair HP and NeoMTA Plus, whereas at 48 and 72 hours, Biodentine showed higher rates of cell viability than MTA Repair HP and NeoMTA Plus ( $P<0.001$ ). The new pulp-capping materials MTA Repair HP, NeoMTA Plus, and Biodentine showed a suitable degree of cytocompatibility with hDPSCs.
10	Sultana et al	2018	ProRoot MTA (MTA; Dentsply Tulsa Dental, Tulsa, OK), Biodentine (BD; Septodont, Saint Maur de Fosses, France)	human bone marrow derived mesenchymal stem cells (hBMSCs)	formulated into discs	1 day 3 day 5 day 7 day	MTT assay	MTA have shown the best biocompatibility among the tricalcium silicate materials used with no significant difference between them
11	Sismanoglu, S. Ercal, P.	2022	Biodentine, NeoMTA Plus	tooth germ-derived stem cells (TGSCs)	-	7 day	MTS cell proliferation assay	TGSCs remained viable after 7 days of incubation with all tested materials.
12	Samyuktha et al	2014	mineral trioxide aggregate (MTA), and Biodentine	human periodontal ligament fibroblasts	-	48 hr 72 hr	trypan blue dye assay+inverted phase contrast microscope	Mineral trioxide aggregate was shown to be less toxic to periodontal ligament fibroblasts than Endosequence Root Repair Material and Biodentine. After 24-hour time period, MTA, Endosequence, Biodentine, and control group resulted in cell viabilities of 94.26%, 86.19%, 85.97%, and 96.17%. After 48-hour time period, Endosequence, MTA, Biodentine, and control group resulted in cell viabilities of 82.94%, 82.31%, 81.81%, and 93.34%. Table 2 showed that all the test materials showed no statistical significant difference between them ( $P>0.05$ ).
13	Akbulut et al	2018	Biodentine, Micro-Mega mineral trioxide aggregate (MM-MTA)	human periodontal ligament (hPDL) fibroblasts	1:1 1:2 1:4 1:8	24 hr	XTT assay	Three-day samples were more cytotoxic than 1-day samples ( $P<0.05$ ). Eluates from the cements at 1:1 dilution were significantly more cytotoxic ( $P<0.05$ ). This in vitro study demonstrated that Biodentine and Compomer were more biocompatible than the other root-end filling materials
14	Pinheiro et al	2018	mineral trioxide aggregate (MTA; Angelus, Londrina, PR, Brazil) and Biodentine (BD; Septodont, Saint-Maur-de-Fosses, France)	Fibroblasts (3T3)	-	-	MTT assay and sulforhodamine B assays	NeoMTA Plus showed a biocompatible behavior similar to mineral trioxide aggregate and Biodentine. The material extracts induced greater viability on fibroblast cells after setting.
15	Pelepenko et al	2021	ProRoot MTA and Biodentine	human fibroblast cells	-	-	MTT assay	White-MTAFlow showed MTT cytocompatibility similarly to the control group. White-MTAFlow in 'thin' consistency presents comparable physicochemical, biological, and antimicrobial properties to ProRoot MTA and Biodentine, and does not cause color alteration in dentin.

16	Pedrosa et al	2022	Biodentine and MTA Repair HP	human dental pulp cells (hDPCs)	1:1, 1:4, and 1:16	24 hr 48 hr 72 hr	MTT assay	Cell viability of LPS-activated hPDCs was higher than untreated control in 48 and 72 h ( $P < 0.05$ ).
17	Pedrosa et al	2022	Biodentine, MTA Repair HP and White MTA	human periodontal ligament stem cells (hPDLSCs)	pure, 1:4 and 1:16	24 hr 48 hr 72 hr	MTT assay	At 24 h, pure extract of MTA Repair HP and Biodentine 1:16 presented higher cell viability compared to control. Lower cell viability was found for pure extract of Cimmo HD, MTA Repair HP 1:4 and 1:16, and White MTA 1:16. At 48 h, pure extract of Bio-C Repair and MTA Repair HP presented higher cell viability compared to control. At 72 h, only the pure extract of MTA Repair HP led to higher cell proliferation compared to control.
18	Pedano et al	2018	Nex-Cem MTA (GC), Biodentine (Septodont)	Human dental pulp cells	(100%, 50%, 25% and 10%)	-	XTT-colorimetric assay	all three cements stimulated proliferation, migration and odontogenic differentiation of HDPCs.
19	Margunato et al	2015	ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK), Biodentine (Septodont, Saint Maur des Fossés, France), and MM-MTA (Micro-Mega, Besançon Cedex, France)	human bone marrow stem cells (hBMSCs)	-	days 1, 3, 7, and 14	3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulfo-phenyl)-2H tetrazolium assay	MTA, Biodentine, and MM-MTA did not exhibit a cytotoxic effect on hBMSCs after 14 days in culture.
20	Donghee et al	2020	ProRoot MTA (Dentsply Tulsa Dental Specialties), Biodentine (Septodont), and RetroMTA (BioMTA)	human gingiva-derived stem cells (GDSCs)	-	-	MTT assay	In the MTT assay, no significant differences were found among the ProRoot MTA, Biodentine, and control groups during the test period ( $p > 0.05$ ). The Endocem Zr and RetroMTA groups showed relatively lower cell viability than the control group at day 7 ( $p < 0.05$ ).
21	Jovanovic et al	2014	MTA and Biodentine	permanent fibroblast cell lines (MRC-5 and L929)	-	24 hr 48 hr	MTT assay	MTT test showed that after 24 and 48 hours in both cell cultures, Biodentine had the largest percentage of cytotoxicity. The lowest percentage of cytotoxicity showed MTA in both groups.
22	Hengameh, et al	2014	CEM cemen and MTA	human mesenchymal stem cells (hMSCs)	-	-	MTT assay	Cell viability was not significantly different. CEM cement has acceptable toxicity.
23	Gomes-Cornelio et al	2017	Biodentine (BIO, Septodont), MTA Plus (MTA P, Avalon)	human osteoblastic cells (Saos-2)	-	-	MTT assay	Results All tested cements showed dose-dependent responses in cell viability (MTT). All materials had suitable biocompatibility and bioactivity. The MTA P, BIO and CSCR ZrO <sub>2</sub> groups had the highest viability rates.

24	Assadian et al	2022	OrthoMTA (OMTA), Biodentine (BD) and Calcium-Enriched Mixture (CEM)	human dental pulp stem cells (hDPSCs)	10, 25, 50 and 100%	Days 1, 3 and 5	-	Optimal cell proliferation was observed in all groups, regardless of concentration and time-point.
25	Dahake et al	2020	MTA (Mineral Trioxide Aggregate) and Biodentine	Stem Cells from Human Exfoliated Deciduous teeth (SHED)	-	7 day	MTT assay	It was observed that EMD imparted the highest cell viability at the end of 7 days ( $p < 0.001$ ) followed by Biodentine and MTA.
26	Costa et al	2016	ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK), MTA Plus (Prevest Denpro Limited, Jammu City, India), MTA Fillapex (Angelus, Londrina, PR, Brazil), and Biodentine (Septodont, Saint-Maur-des-Fosses, France)	human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs)	(1:2-1:20)	-	-	Diluted extracts from MTA ProRoot and MTA Plus had evident stimulatory effects on the proliferation of hMSCs. MTA Fillapex exhibited the highest toxicity in hMSCs and HUVECs.
27	Collado-Gonzalez et al	2017	Biodentine (Septodont, Saint-Maur-des-Fosses, France) MTA (Angelus, Londrina, PR, Brazil)	stem cells isolated from human exfoliated primary teeth (SHEDs)	-	24, 48 and 72h	MTT assay	Biodentine exhibited better cytocompatibility and bioactivity than MTA Angelus, TheraCal LC and IRM.
28	Bossù, M. Mancini, P.	2021	ProRoot MTA and Biodentine	Human osteogenic sarcoma (Saos-2) cells	-	-	-	Cell viability assay demonstrated that Saos-2 cells had a dose- and time-dependent cytotoxicity to both analyzed cements, although cells exposed to ProRoot MTA showed a better cell vitality than those exposed to Biodentine ( $p < 0.001$ ).
29	Bortoluzzi et al	2015	MTA Angelus and Biodentine	human dental pulp stem cells (hDPSCs)	-	-	XTT assay and flow cytometry	The cytotoxic effects of Biodentine and TheraCal LC on hDPSCs were time- and concentration-dependent.
30	Birant et al	2021	NeoMTA Plus, ProRootMTA and Biodentine	Dental pulp stem cells (DPSCs)	-	Days 1, 3 and 7	flow cytometry using an Annexin V detection kit	All groups showed cell viability similar to that of the control group on 1st, 3rd and 7th d. Although Biodentine exhibited higher cell viability rates than the other material groups, no statistically significant differences were noted between the sampled days ( $p > 0.05$ ). All materials extracts are not cytotoxic.
31	Attik et al	2014	Biodentine(TM) and White ProRoot(R) mineral trioxide aggregate (MTA(R))	MG63 osteoblast-like cells	-	Days 1, 3 and 5	MTT assay	The MTT test for Biodentine(TM) was similar to that of MTA(R).
32	Agrafioti et al	2016	Biodentine and MTA	dental pulp stem cells (DPSCs)	-	4 day 7 day	MTT assay	Biodentine expressed significantly higher cell viability compared with all other groups after 4 days, with no differences after 7 days. Notably, cell viability was significantly greater in 24-hour set MTA compared with 1-hour set MTA and control groups after 7 days.

Table 4. Checking the quality of articles based on the ESA Protocol checklist\*

Author	Screening question		Sample Selection & Experimental Setup				Reagents & Cells							Sample Size		Random Allocation to groups	Blinding Allocation Concealment and Outcome Assessment	Attrition/Exclusions			score
	Q1	Q2	Q31	Q32	Q33	Q34	Q41	Q42	Q43	Q44	Q45	Q46	Q47	Q51	Q52	Q6	Q7	Q81	Q82	Q83	
Khedmat et al. [4]	0	-	-	1	1	0.5	1	0	-	1	1	1	-	1	1	-	-	-	-	-	85/11
Omid et al. [43]	1	-	-	1	1	1	1	0	-	1	1	1	0	1	1	-	-	-	-	-	10/11
Saberi et al. [44]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Saberi et al. [45]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Küçükkaya et al. [46]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Zhou et al. [6]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Youssef et al. [47]	0	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9/11
Weekate et al. [48]	1	-	-	1	1	0.5	1	0	-	1	1	1	-	1	1	-	-	-	-	-	95/11
Tomas Catal et al. [49]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Sultana et al. [50]	0	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9/11
Sismanoglu et al. [51]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Samyuktha et al. [52]	0	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9/11
Akbulut et al. [53]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Pinheiro et al. [54]	1	-	-	1	1	0.5	1	1	-	1	1	1	-	1	1	-	-	-	-	-	105/11
Pelepenko et al. [55]	0	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9/11
Pedorsa et al. [56]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Pedorsa et al. [57]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Pedano et al. [58]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Margunato et al. [59]	1	-	-	1	1	0.5	1	0	-	1	1	1	-	1	1	-	-	-	-	-	95/11
Lee [60]	0	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9/11
Jovanovic et al. [61]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Hengamch et al. [62]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Gomes-Corn [63]	1	-	-	1	1	0.5	1	1	-	1	1	1	-	1	1	-	-	-	-	-	105/11

ASSADIAN <i>et al.</i> [64]	1	-	-	1	1	0.5	1	0	-	1	1	1	-	1	1	-	-	-	-	-	9.5/11
Dahake <i>et al.</i> [42]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Costa <i>et al.</i> [65]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Collado-Gonz <i>et al.</i> [66]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Bossù <i>et al.</i> [67]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11
Bortoluzzi <i>et al.</i> [68]	1	-	-	1	1	0.5	1	1	-	1	1	1	-	1	1	-	-	-	-	-	10.5/11
Birant <i>et al.</i> [69]	1	-	-	1	1	1	1	0	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Attik <i>et al.</i> [70]	0	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	10/11
Agrafioti <i>et al.</i> [71]	1	-	-	1	1	1	1	1	-	1	1	1	-	1	1	-	-	-	-	-	11/11

Description: Yes=1, No=0, Partially=0.5, Not applicable=-

**Table 5.** Characteristics of the studies included in the meta-analysis

Row	Names	Type of calcium silicate base cement (MTA OR Biodentin OR Cem cement)	Type of cell line used	Substance concentration	Cell exposure time	Measurement method	significant or not significant
1	Omidi <i>et al.</i> [43]	Angelus mineral trioxide aggregate (MTA; Angelus), calcium-enriched mixture (CEM; BioniqueDent), and Biodentine (Septodont)	human dental pulp stem cells (hDPSCs)	Non diluted 1:2 1:4	24 hr 48 hr 72 hr	MTT assay	No significant effect at 48 h; significant increase in cell proliferation at 72 h for both CEM ( $P<0.01$ ) and Biodentine ( $P<0.05$ ). Biodentine induced significantly higher TGF- $\beta$ 1 secretion than CEM ( $P<0.05$ ).
2	Youssef <i>et al.</i> [47]	mineral trioxide aggregate (MTA), Biodentine	dental pulp stem cells (DPSCs)	Non diluted	3 day	MTT assay	All materials showed significant cytotoxicity compared with control at day 3 ( $P<0.0001$ ); Biodentine showed significantly lower cell viability than MTA.
3	Tomas-Catala <i>et al.</i> [49]	MTA Repair HP, NeoMTA Plus, and Biodentine	human dental pulp stem cells (hDPSCs)	Non diluted 1:2 1:4	24 hr 48 hr 72 hr	MTT assay	Biodentine showed significantly higher cell viability than MTA Repair HP and NeoMTA Plus at 48 and 72 h; highest cell migration rate observed with Biodentine ( $P<0.001$ )
4	Assadian <i>et al.</i> [64]	OrthoMTA (OMTA), Biodentine (BD) and Calcium-Enriched Mixture (CEM)	human dental pulp stem cells (hDPSCs)	Non diluted 1:2 1:4	Days 1, 3 and 5	MTT assay	Not significant for cell viability at days 1 and 3; significant reduction only for 100% Biodentine at day 5 ( $P=0.016$ ).
5	Agrafioti <i>et al.</i> [71]	Biodentine and MTA	dental pulp stem cells (DPSCs)	Non diluted	4 day 7 day	MTT assay	Biodentine showed significantly higher cell viability than MTA at 4 days ( $P<0.01$ ); no significant difference between Biodentine and MTA at 7 days ( $P>0.05$ ).
6	Saberi <i>et al.</i> [45]	mineral trioxide aggregate (MTA), calcium-enriched mixture (CEM) cement, Biodentine (BD)	human gingival fibroblasts (HGFs)	Non diluted	24 hr 48 hr 168 hr	MTT assay	No significant difference versus control at 24 and 48 h; significant reduction in cell viability for MTA ( $P<0.05$ ) and Biodentine ( $P<0.01$ ) at 168 h

7	Zhou et al. [6]	Biodentine, White Pro Root mineral trioxide aggregate (MTA)	human gingival fibroblasts	1:1 1:2 1:4	1 day 3 day 7 day	flow cytometry	No significant difference in cell viability between Biodentine and MTA at any concentration or time point ( $P>0.05$ ); both significantly less cytotoxic than glass ionomer cement ( $P<0.05$ ).
8	Dahake et al. [42]	MTA (Mineral Trioxide Aggregate) and Biodentine	Stem Cells from Human Exfoliated Deciduous Teeth (SHED)	Non diluted	7 day	MTT assay	Biodentine and MTA showed significantly higher cell viability compared with control at 7 days; Biodentine showed higher viability than MTA ( $P<0.001$ ).
9	Collado-Gonzalez et al. [66]	Biodentine (Septodont, Saint-Maur-des-Fosses, France) MTA (Angelus, Londrina, PR, Brazil)	stem cells isolated from human exfoliated primary teeth (SHEDs)	Non diluted 1:2 1:4	24, 48 and 72h	MTT assay	Biodentine significantly increased cell viability compared with control and MTA from 48 h onward ( $P<0.01$ ).
10	Margunato et al. [59]	ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK), Biodentine (Septodont, Saint Maur des Fossés, France), and MM-MTA (Micro-Mega, Besançon Cedex, France)	human bone marrow stem cells (hBMSCs)	Non diluted	days 1, 3, 7, and 14	MTS assay	No cytotoxic effects observed for any material; all materials significantly stimulated osteogenic differentiation compared with control ( $P<0.05$ ).
11	Sultana et al. [50]	ProRoot MTA (MTA; Dentsply Tulsa Dental, Tulsa, OK), Biodentine (BD; Septodont, Saint Maur de Fosses, France)	human bone marrow-derived mesenchymal stem cells (hBMSCs)	Non diluted	1 day 3 day 5 day 7 day	MTT assay	ERRM and MTA showed significantly higher osteogenic activity than Biodentine ( $P<0.05$ ); no significant difference between ERRM and MTA in cell viability ( $P>0.05$ ).

#### **The results of the meta-analysis of MTA cytotoxicity in different concentrations, times, and cell lines were reported as follows:**

As shown in the forest plot analyses, different results were observed among studies regarding the effect of undiluted MTA on cell viability at different exposure times (24, 48, and 72 hours, as well as 5 and 7 days). Overall, the meta-analysis demonstrated no significant difference in the survival of cells exposed to undiluted MTA compared with the control group after 24 hours (SMD  $-0.39$ ; 95% CI  $-1.38$  to  $0.60$ ;  $P=0.44$ ) ( $I^2$  74.25%;  $P<0.001$ ), 72 hours (SMD  $0.30$ ; 95% CI  $-0.96$  to  $1.56$ ;  $P=0.64$ ) ( $I^2$  79.55%;  $P<0.001$ ), 5 days (SMD  $-0.13$ ; 95% CI  $-1.01$  to  $0.75$ ;  $P=0.77$ ) ( $I^2$  0.00%;  $P=0.74$ ), and 7 days (SMD  $0.74$ ; 95% CI  $-1.13$  to  $2.61$ ;  $P=0.44$ ) ( $I^2$  88.41%;  $P<0.001$ ).

However, a statistically significant difference was observed at 48 hours, where cell viability was significantly lower in the undiluted MTA group compared with the control group (SMD  $-2.11$ ; 95% CI  $-4.14$  to  $-0.08$ ;  $P=0.04$ ) ( $I^2$  80.8%;  $P=0.01$ ), indicating a cytotoxic effect at this time point (Fig. 2).

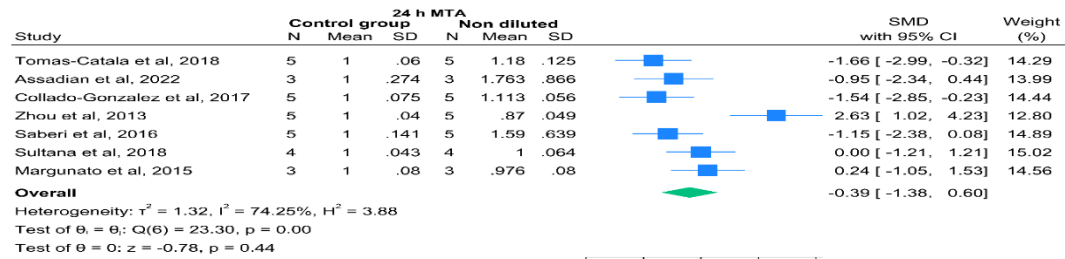
As shown in the forest plot analyses, different results were observed among studies regarding the effect of MTA at a concentration of 1:2 on cell viability at different exposure times (24, 48, and 72 hours). The meta-analysis demonstrated a borderline significant increase in cell viability at 24 hours compared with the control group (SMD  $0.90$ ; 95% CI  $-0.00$  to  $1.81$ ;  $P=0.05$ ) ( $I^2$  50.26%;  $P=0.11$ ). In contrast, no statistically

significant difference in cell viability was observed at 48 hours (SMD  $-2.20$ ; 95% CI  $-6.98$  to  $2.59$ ;  $P=0.37$ ) ( $I^2$  92.53%;  $P<0.001$ ) or at 72 hours (SMD  $-0.21$ ; 95% CI  $-1.02$  to  $0.59$ ;  $P=0.61$ ) ( $I^2$  44.09%;  $P=0.15$ ) compared with the control group (Fig. 3).

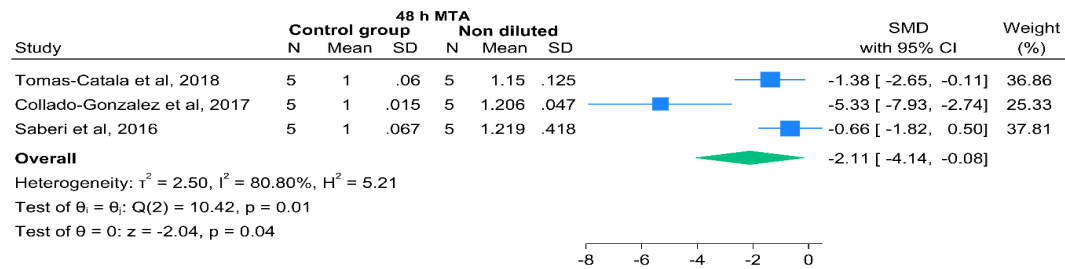
As shown in the forest plot analyses, the effect of MTA at a concentration of 1:4 on cell viability was evaluated at different exposure times (24, 48, and 72 hours). The meta-analysis demonstrated no statistically significant difference in cell viability between the MTA (1:4) and control groups after 24 hours of exposure (SMD  $0.25$ ; 95% CI  $-0.34$  to  $0.84$ ;  $P$ -value 0.40) ( $I^2$  0.00%;  $P$ -value 0.57). Similarly, no significant difference was observed at 48 hours (SMD  $-0.99$ ; 95% CI  $-3.36$  to  $1.38$ ;  $P$ -value 0.41) ( $I^2$  84.49%;  $P$ -value 0.01). At 72 hours, cell viability also did not differ significantly between the groups (SMD  $0.55$ ; 95% CI  $-0.44$  to  $1.53$ ;  $P$ -value 0.28) ( $I^2$  59.58%;  $P$ -value 0.06) (Fig. 4).

#### **The results of the meta-analysis of Biodentine cytotoxicity in different concentrations, times, and cell lines were reported as follows:**

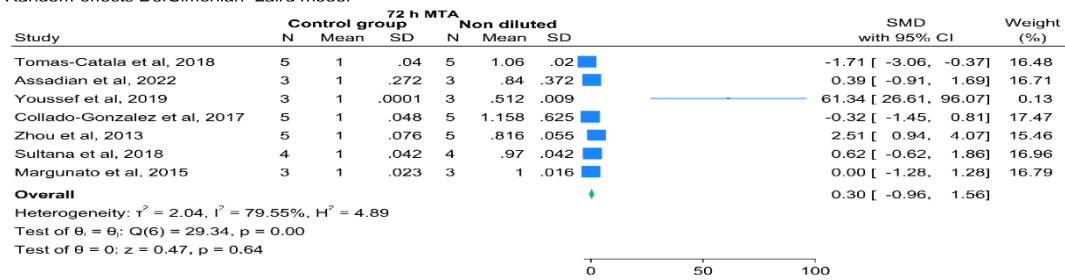
As shown in the forest plot analyses, the effect of undiluted Biodentine on cell viability was evaluated at different exposure times (24, 48, and 72 hours, as well as 5 and 7 days). The meta-analysis demonstrated no statistically significant difference in cell viability between the undiluted Biodentine and control groups after 24 hours of exposure (SMD  $-0.09$ ; 95% CI  $-1.77$  to  $1.59$ ;  $P$ -value 0.92) ( $I^2$  85.75%;  $P$ -value  $<0.001$ ). Similarly, no



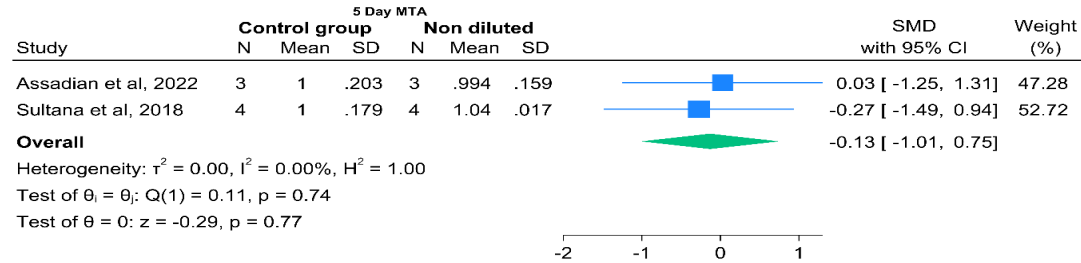
Random-effects DerSimonian-Laird model



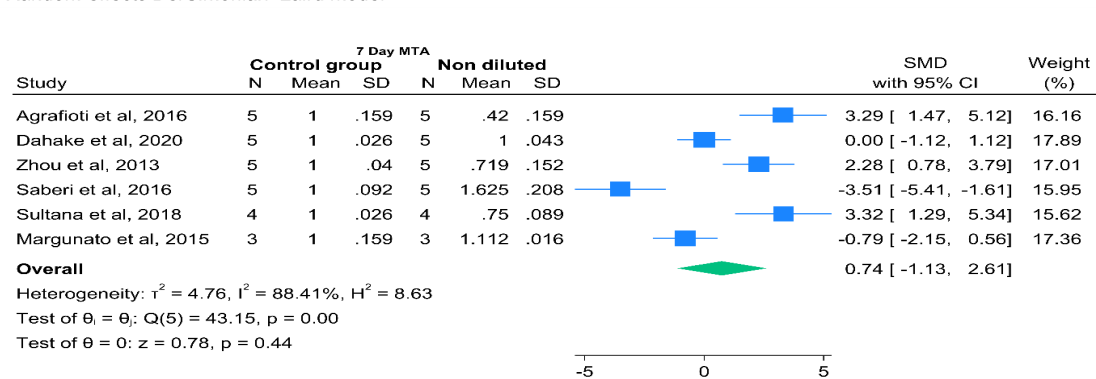
Random-effects DerSimonian-Laird model



Random-effects DerSimonian-Laird model

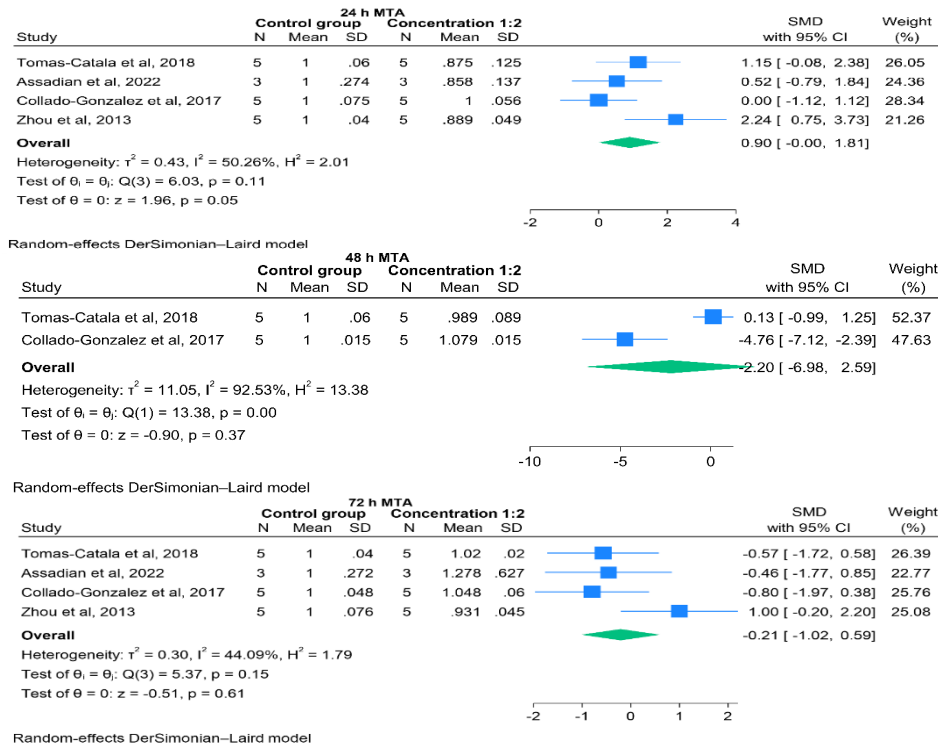


Random-effects DerSimonian-Laird model

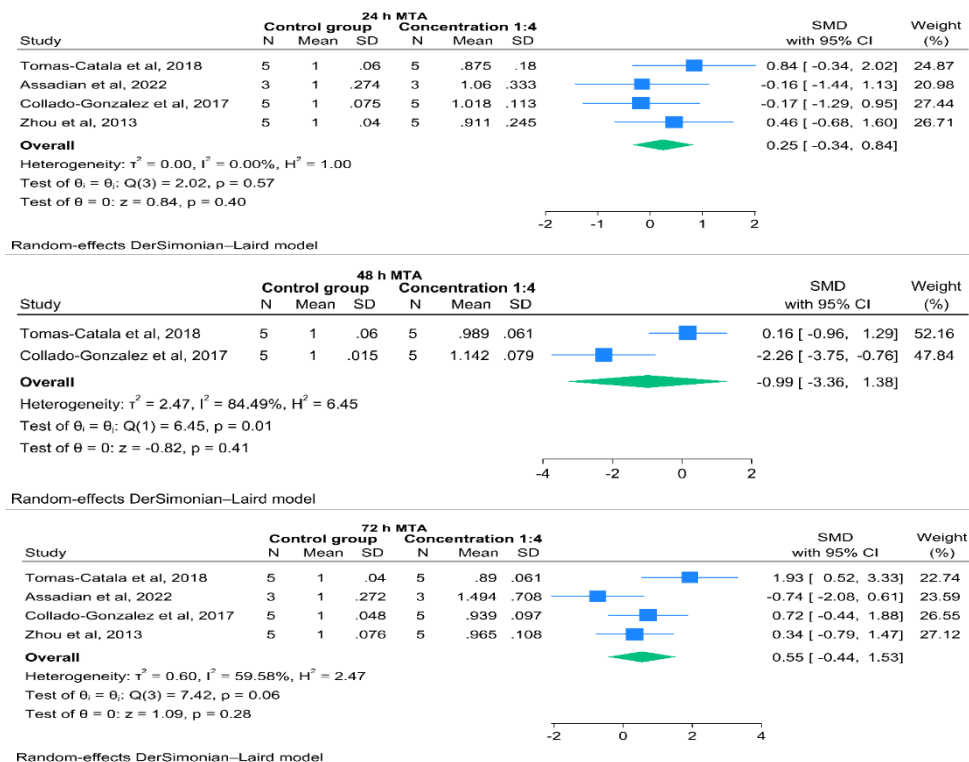


Random-effects DerSimonian-Laird model

**Figure 2.** Forest plot of studies evaluating cell viability following exposure to undiluted MTA at different time intervals (24, 48, and 72 hours, as well as 5 and 7 days). The meta-analysis demonstrated a statistically significant reduction in cell viability at 48 hours compared with the control group ( $P=0.01$ ), indicating a cytotoxic effect at this time point. In contrast, no significant differences in cell viability were observed at 24 and 72 hours, or after 5 and 7 days of exposure (95% CI).



**Figure 3.** Forest plot of studies evaluating cell viability following exposure to MTA at a concentration of 1:2 at different time intervals (24, 48, and 72 hours). No statistically significant differences in cell viability were observed between the MTA (1:2) and control groups at any of the evaluated time points (24, 48, and 72 hours) (95% CI)



**Figure 4.** Forest plot of studies evaluating cell viability following exposure to MTA at a concentration of 1:4 at different time intervals (24, 48, and 72 hours). No statistically significant differences in cell viability were observed between the MTA (1:4) and control groups at any of the evaluated time points (24, 48, and 72 hours) (95% CI).

significant differences were observed at 48 hours (SMD  $-1.71$ ; 95% CI  $-4.37$  to  $0.96$ ;  $P$ -value  $0.21$ ) ( $I^2$  91.05%;  $P$ -value  $<0.001$ ) or at 72 hours (SMD  $-1.75$ ; 95% CI  $-4.99$  to  $1.49$ ;  $P$ -value  $0.29$ ) ( $I^2$  92.16%;  $P$ -value  $<0.001$ ).

In addition, no statistically significant difference in cell viability was found after 5 days of exposure (SMD  $0.73$ ; 95% CI  $-0.52$  to  $1.97$ ;  $P$ -value  $0.25$ ) ( $I^2$  40.10%;  $P$ -value  $0.20$ ) or after 7 days (SMD  $0.15$ ; 95% CI  $-1.58$  to  $1.89$ ;  $P$ -value  $0.86$ ) ( $I^2$  86.50%;  $P$ -value  $<0.001$ ) compared with the control group (Fig. 5).

As shown in the forest plot analyses, the effect of Biodentine at a concentration of 1:2 on cell viability was evaluated at different exposure times (24, 48, and 72 hours). The meta-analysis demonstrated no statistically significant difference in cell viability between the Biodentine (1:2) and control groups after 24 hours of exposure (SMD  $-0.11$ ; 95% CI  $-0.70$  to  $0.48$ ;  $P$ -value  $0.71$ ) ( $I^2$  0.00%;  $P$ -value  $0.40$ ). Similarly, no significant difference was observed at 48 hours (SMD  $-5.66$ ; 95% CI  $-14.50$  to  $3.18$ ;  $P$ -value  $0.21$ ) ( $I^2$  92.36%;  $P$ -value  $<0.001$ ). At 72 hours, the difference in cell viability between the two groups was also not statistically significant (SMD  $-4.06$ ; 95% CI  $-8.27$  to  $0.14$ ;  $P$ -value  $0.06$ ) ( $I^2$  93.27%;  $P$ -value  $<0.001$ ) (Fig. 6).

As shown in the forest plot analyses, the effect of Biodentine at a concentration of 1:4 on cell viability was evaluated at different exposure times (24, 48, and 72 hours). The meta-analysis indicated no statistically significant difference in cell viability between the Biodentine (1:4) and control groups after 24 hours of exposure (SMD  $-0.55$ ; 95% CI  $-1.39$  to  $0.29$ ;  $P$ -value  $0.20$ ) ( $I^2$  46.33%;  $P$ -value  $0.13$ ).

In contrast, at 48 hours, a statistically significant reduction in cell viability was observed in the Biodentine (1:4) group compared to the control group (SMD  $-3.90$ ; 95% CI  $-6.85$  to  $-0.94$ ;  $P$ -value  $0.01$ ) ( $I^2$  72.28%;  $P$ -value  $0.06$ ). At 72 hours, although the pooled effect size suggested a decrease in cell viability in the Biodentine (1:4) group, the difference was not statistically significant (SMD  $-0.83$ ; 95% CI  $-2.43$  to  $0.76$ ;  $P$ -value  $0.31$ ) ( $I^2$  81.95%;  $P$ -value  $<0.001$ ) (Fig. 7).

#### **The results of the meta-analysis of the cytotoxicity of CEM cement in different concentrations, times, and cell lines were reported as follows:**

As shown in the forest plot analyses, the effect of non-diluted CEM cement on cell viability was evaluated at different exposure times (24, 48, and 72 hours). The meta-analysis revealed no statistically significant difference in cell viability between the non-diluted CEM cement and control groups after 24 hours of exposure (SMD  $-0.39$ ; 95% CI  $-1.89$  to  $1.11$ ;  $P$ -value  $0.61$ ) ( $I^2$  64.52%;  $P$ -value  $0.09$ ) (Fig. 8).

Similarly, at 48 hours, no statistically significant difference in cell viability was detected between the non-diluted CEM cement

and control groups (SMD  $0.31$ ; 95% CI  $-0.49$  to  $1.11$ ;  $P$ -value  $0.45$ ) ( $I^2$  0.00%;  $P$ -value  $0.43$ ). At 72 hours, although the pooled effect size suggested a reduction in cell viability in the non-diluted CEM cement group compared to the control group, this difference was not statistically significant (SMD  $-2.80$ ; 95% CI  $-8.62$  to  $3.01$ ;  $P$ -value  $0.34$ ) ( $I^2$  92.92%;  $P$ -value  $<0.001$ ).

#### **Sensitivity test**

Fig. 9 presents the results of the leave-one-out sensitivity analyses performed to evaluate the robustness of the pooled effect estimates in cytotoxicity studies. At 24 hours, sequential omission of individual studies resulted in pooled standardized mean differences (SMDs) ranging from  $-0.18$  to  $-0.82$ . With the exception of one exclusion scenario, all recalculated confidence intervals crossed the null value, indicating that the overall effect estimate was not substantially influenced by any single study.

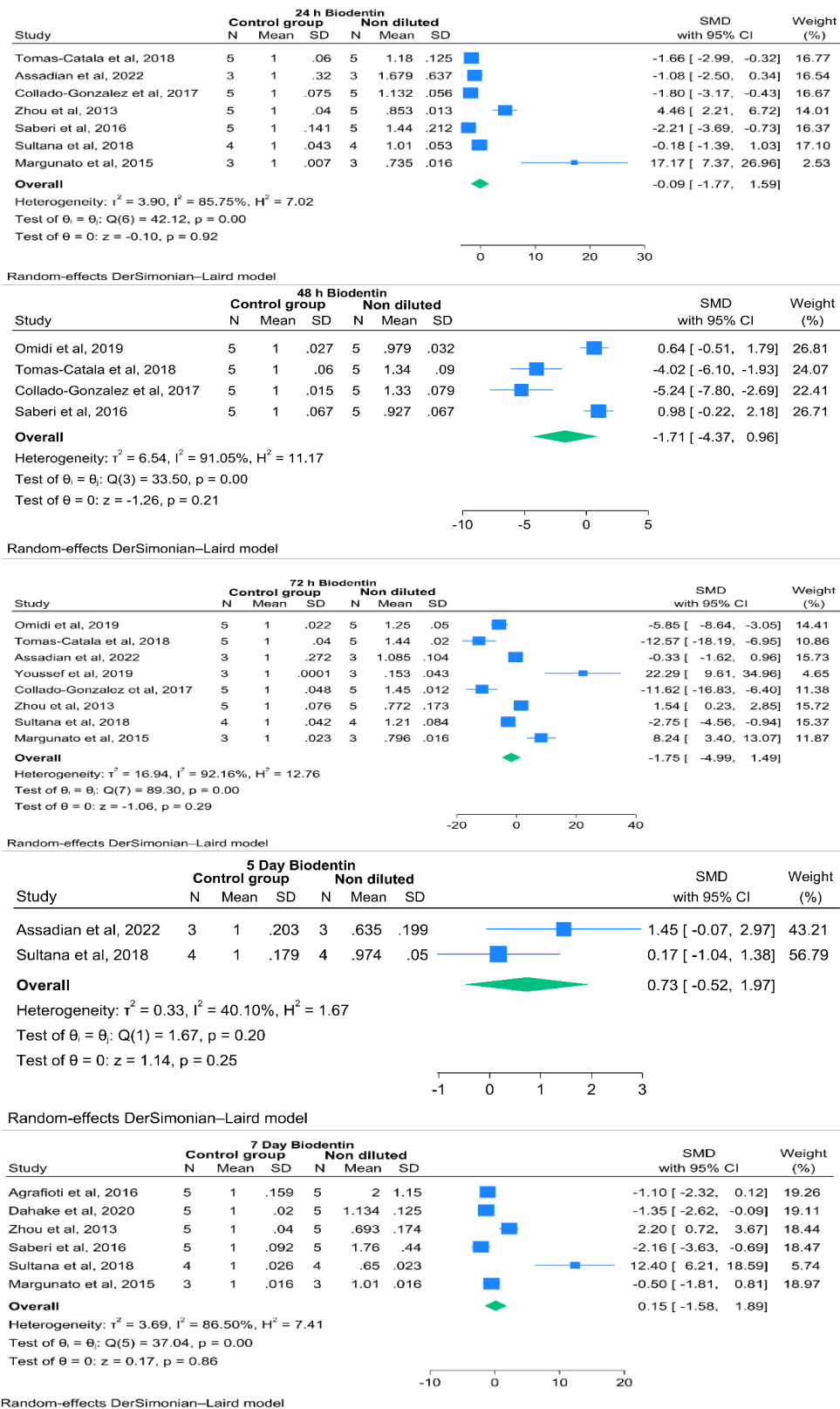
At 48 hours, sensitivity analysis demonstrated that the pooled SMDs varied between  $-0.99$  and  $-3.19$  following exclusion of individual studies. Although omission of one study led to a statistically significant pooled estimate, the direction of the effect remained consistent, suggesting that the overall findings were not driven by a single influential study.

Similarly, at 72 hours, the recalculated pooled SMDs ranged from  $-0.12$  to  $0.68$ , with all corresponding 95% confidence intervals including zero. At the longest follow-up interval, sequential exclusion of studies yielded pooled SMDs ranging from  $0.25$  to  $1.51$ , none of which resulted in a statistically significant change in the overall effect estimate.

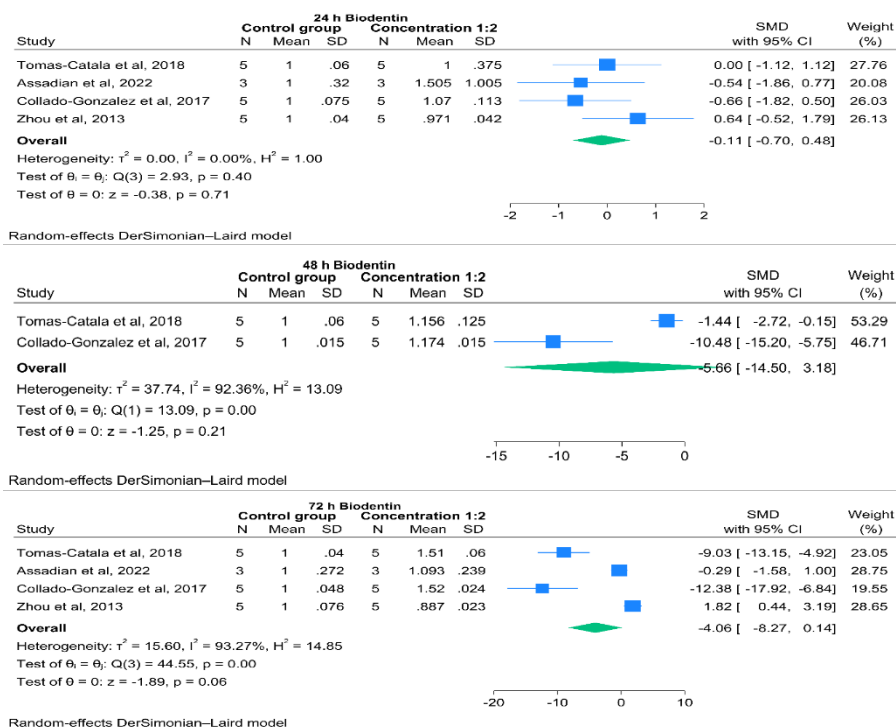
Overall, the sensitivity analyses confirmed that the pooled results remained stable across all time intervals and were not disproportionately affected by any individual study, supporting the robustness and reliability of the meta-analysis findings.

Fig. 10 illustrates the results of the leave-one-out sensitivity analyses conducted to evaluate the stability of the pooled effect estimates. At the first evaluated time point, sequential omission of individual studies resulted in pooled standardized mean differences (SMDs) ranging from  $0.52$  to  $1.25$ . Exclusion of one study led to a statistically significant pooled estimate; however, the direction of the effect remained consistent across all omission scenarios, indicating that the overall result was not solely driven by a single study.

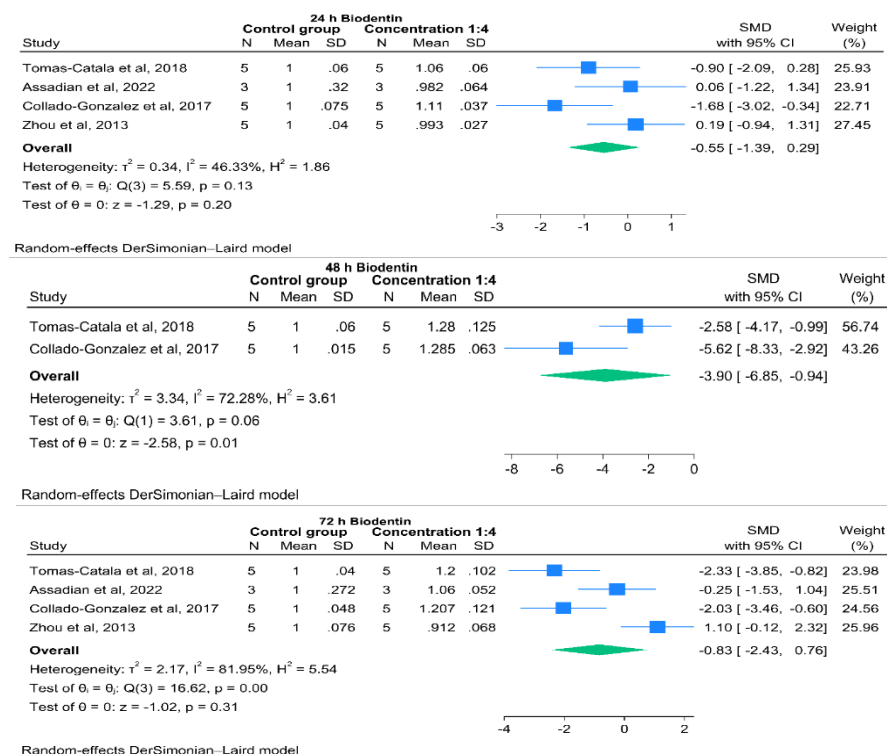
At the subsequent time point, leave-one-out analysis showed that the pooled SMDs ranged from  $-0.62$  to  $-0.01$  following exclusion of individual studies. In all scenarios, the corresponding 95% confidence intervals included the null value, suggesting that removal of any single study did not result in a statistically significant change in the overall effect estimate.



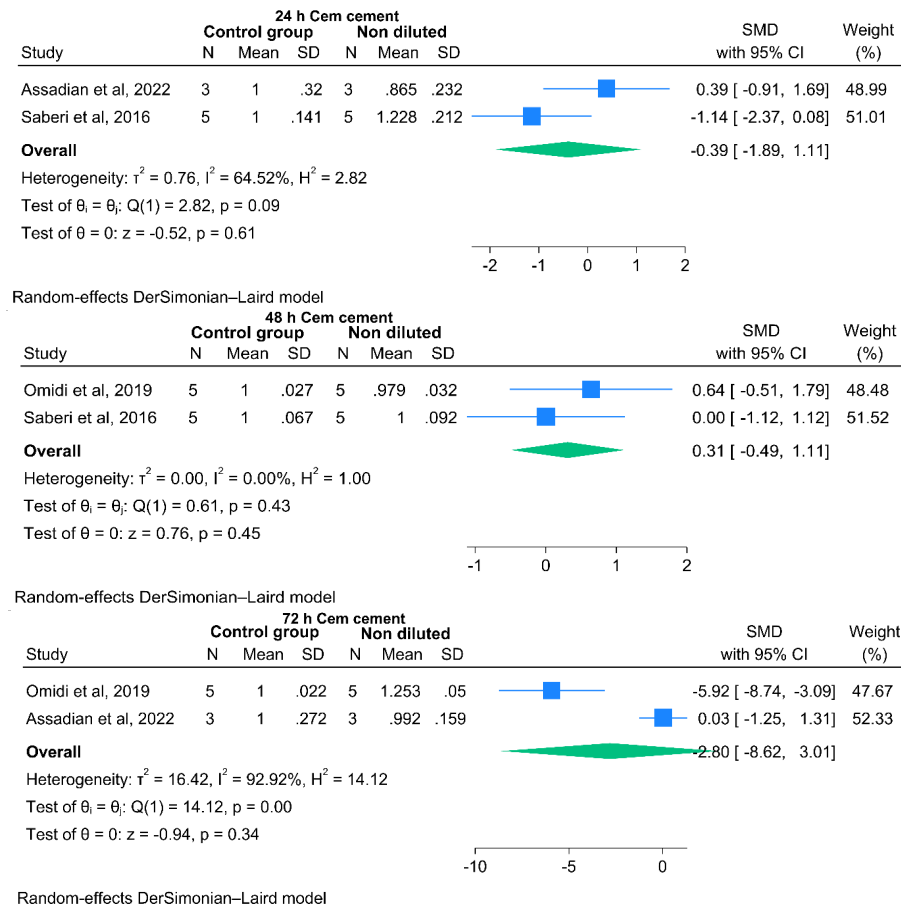
**Figure 5.** Forest plot of studies evaluating cell viability following exposure to undiluted Biodentine at different time intervals (24, 48, and 72 hours, as well as 5 and 7 days). No statistically significant differences in cell viability were observed between the undiluted Biodentine and control groups at any of the evaluated time points (95% CI).



**Figure 6.** Forest plot of studies evaluating cell viability following exposure to Biodentine at a concentration of 1:2 at different time intervals (24, 48, and 72 hours). No statistically significant differences in cell viability were observed between the Biodentine (1:2) and control groups at any of the evaluated time points (95% CI).



**Figure 7.** Forest plot of studies evaluating cell viability following exposure to Biodentine at a concentration of 1:4 at different time intervals (24, 48, and 72 hours). A statistically significant reduction in cell viability was observed at 48 hours, while no significant differences were detected at 24 and 72 hours compared with the control group (95% CI).



**Figure 8.** Forest plot of studies evaluating cell viability following exposure to non-diluted CEM cement at different time intervals (24, 48, and 72 hours). No statistically significant differences in cell viability were observed between the non-diluted CEM cement and control groups at any of the evaluated time points (95% CI).

Overall, these sensitivity analyses demonstrate that although minor variations in effect size were observed after sequential exclusion of individual studies, the pooled results remained generally stable, supporting the robustness of the meta-analysis findings.

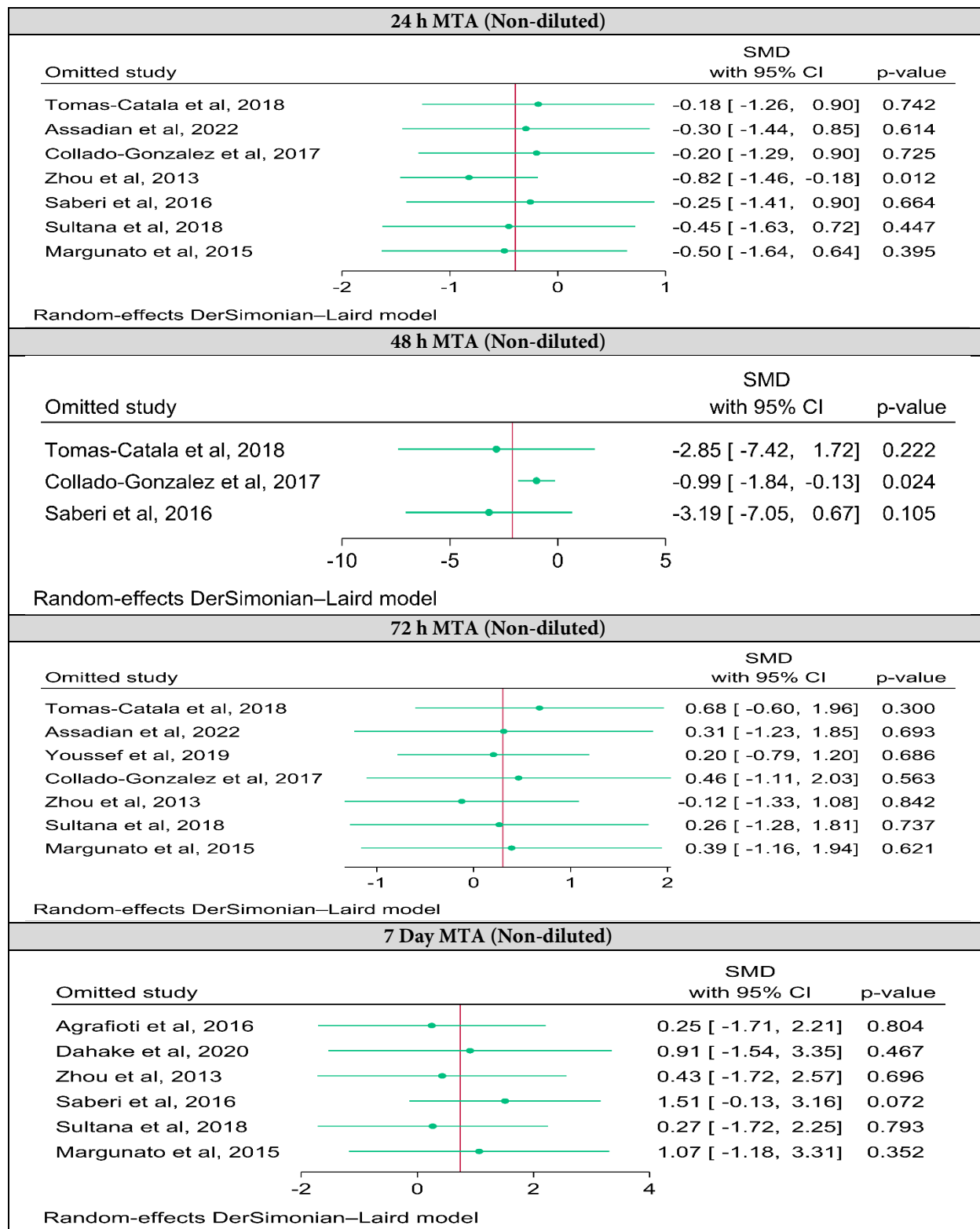
Fig. 11 presents the results of the leave-one-out sensitivity analyses performed to assess the robustness of the pooled effect estimates across different exposure time points. At the first evaluated time point, sequential exclusion of individual studies resulted in pooled standardized mean differences (SMDs) ranging from 0.06 to 0.41. In all omission scenarios, the corresponding 95% confidence intervals crossed the null value, indicating that removal of any single study did not lead to a statistically significant change in the overall effect estimate.

At the subsequent time point, leave-one-out analysis demonstrated that the pooled SMDs ranged from  $-2.76$  to  $-0.63$  following exclusion of individual studies. Despite variations in the magnitude of the effect size, none of the recalculated estimates reached statistical significance, and all confidence

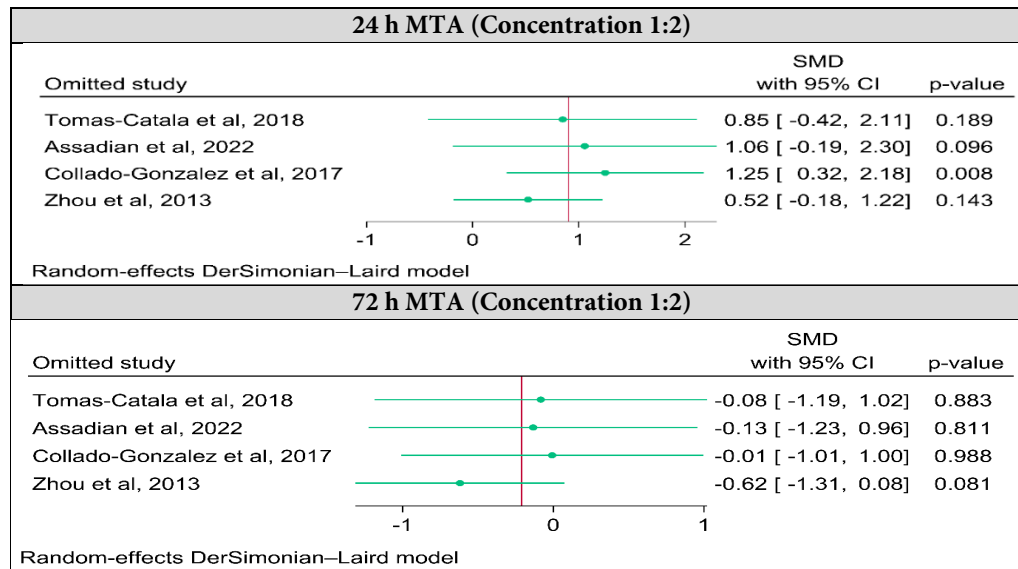
intervals included zero, suggesting stability of the pooled results. At the final evaluated time point, the recalculated pooled SMDs ranged from 0.17 to 0.91. Exclusion of one study resulted in a statistically significant pooled estimate; however, the direction of the effect remained consistent across all omission scenarios, indicating that the overall findings were not disproportionately influenced by any single study.

Overall, these sensitivity analyses demonstrate that the pooled effect estimates were generally stable across all evaluated time points and support the robustness of the meta-analysis findings.

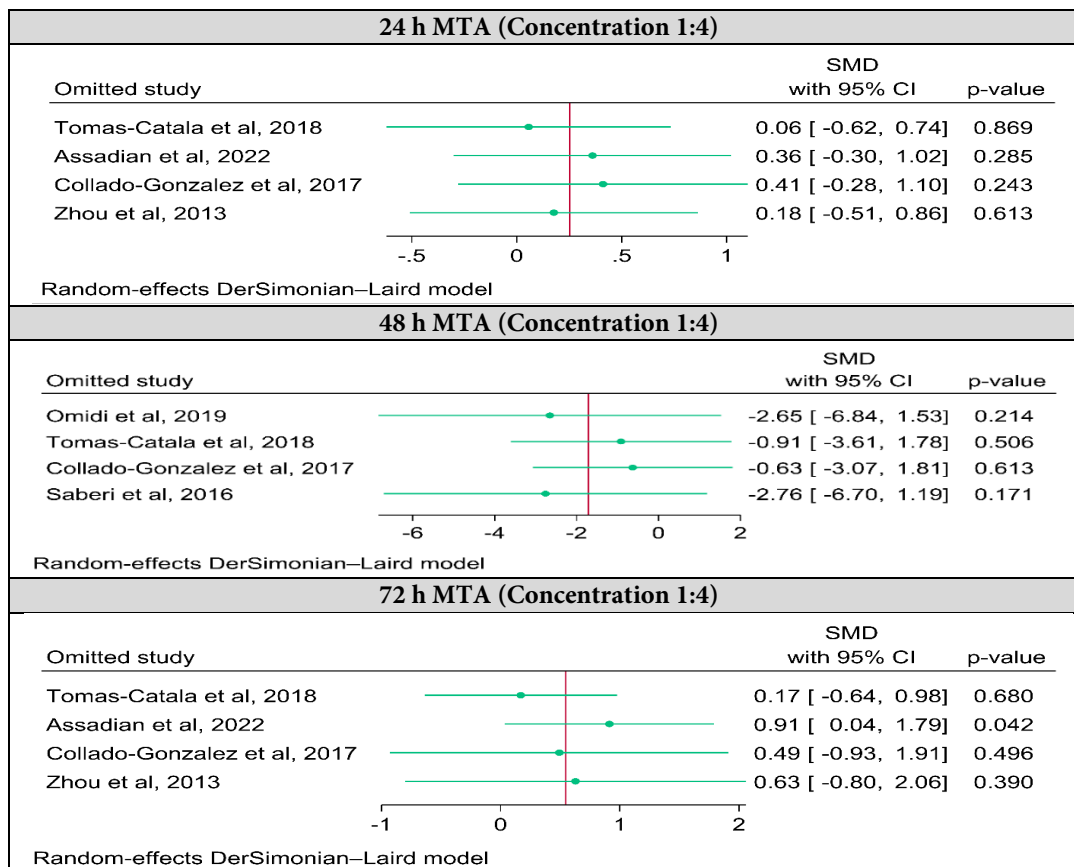
Fig. 12 illustrates the results of the leave-one-out sensitivity analyses conducted to further evaluate the robustness of the pooled effect estimates at different exposure time points. At 24 hours, sequential omission of individual studies resulted in pooled standardized mean differences (SMDs ranging from  $-1.07$  to 0.39), with all corresponding 95% confidence intervals crossing the null value, indicating no statistically significant influence of any single study on the overall effect estimate. At 48 hours, the



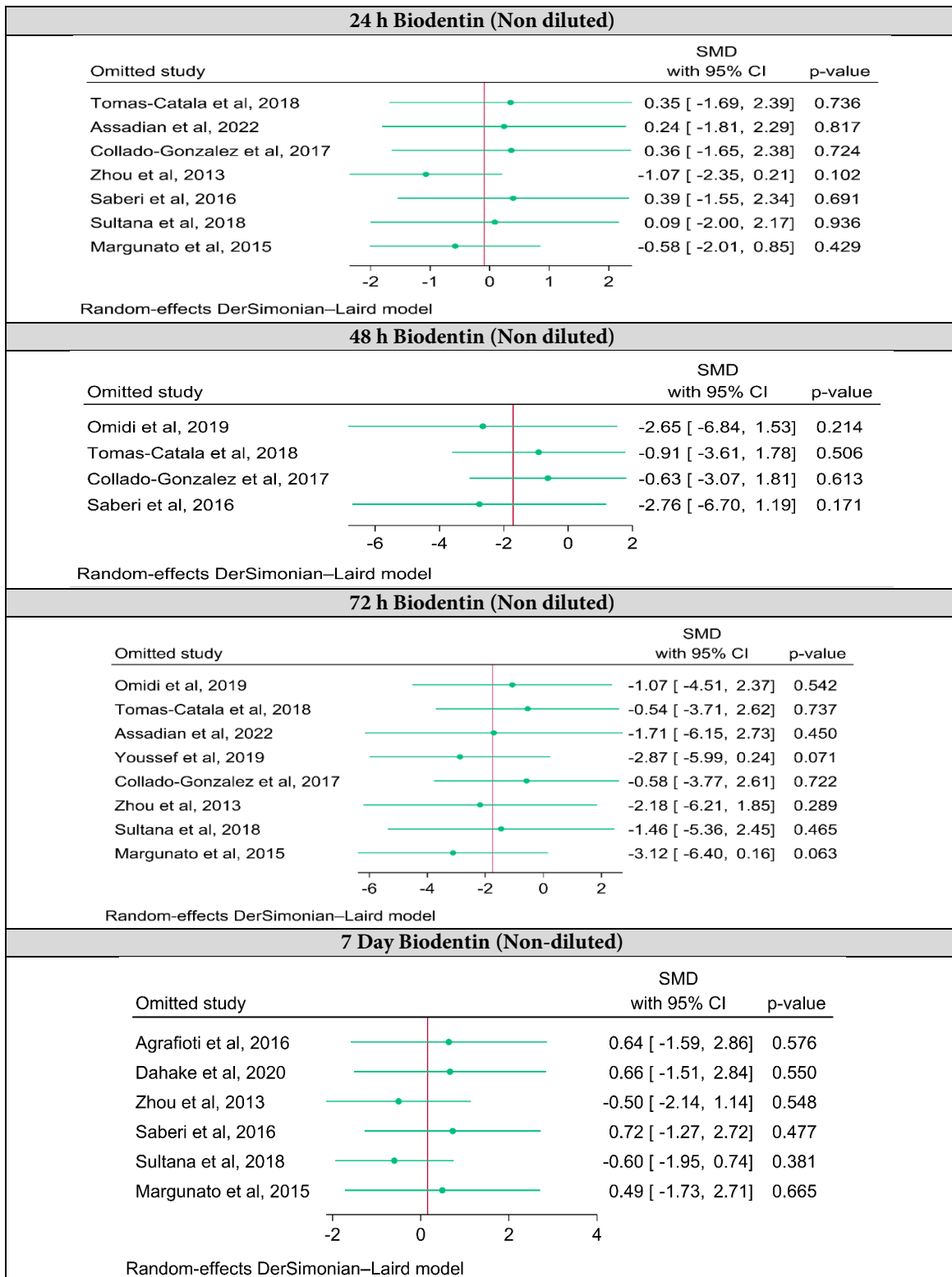
**Figure 9.** Leave-one-out sensitivity analyses assessing the influence of individual studies on the pooled standardized mean difference (SMD) estimates for cytotoxicity outcomes at different time intervals. Each plot illustrates the recalculated pooled effect size following sequential omission of a single study under the random-effects DerSimonian–Laird model. The consistency of effect estimates across exclusion scenarios indicates that the overall results were robust and not driven by any single study (95% CI).



**Figure 10.** Leave-one-out sensitivity analyses showing the influence of individual studies on the pooled standardized mean difference (SMD) estimates under the random-effects DerSimonian–Laird model. Each plot represents the recalculated pooled effect size after sequential omission of one study. The consistency of effect estimates across omission scenarios indicates that the overall findings were not disproportionately affected by any single study (95% CI).



**Figure 11.** Leave-one-out sensitivity analyses illustrating the influence of individual studies on the pooled standardized mean difference (SMD) estimates at different exposure times under the random-effects DerSimonian–Laird model. Each plot represents the pooled effect size recalculated after sequential omission of a single study. The consistency of results across omission scenarios indicates that the overall findings were robust and not driven by any individual study (95% CI).



**Figure 12.** Presents the results of the leave-one-out sensitivity analyses performed to assess the robustness of the pooled effect estimates across different exposure time points. Sequential exclusion of individual studies resulted in only minor variations in the magnitude of the standardized mean differences (SMDs), with no meaningful changes in the direction or statistical significance of the pooled estimates. These findings indicate that the overall results were stable and not driven by any single study.

recalculated pooled SMDs ranged from  $-2.76$  to  $-0.63$  following exclusion of individual studies. Although variations in the magnitude of effect sizes were observed, the confidence intervals consistently included zero, suggesting that the overall findings remained stable and were not driven by any particular study. Similarly, at 72 hours, leave-one-out analyses demonstrated pooled SMDs ranging from  $-3.12$  to  $-0.58$  across exclusion scenarios. Despite slight shifts in effect size magnitude, omission of individual studies did not materially alter the direction or statistical significance of the pooled estimates.

At the longest evaluated time point, the pooled SMDs ranged from  $-0.60$  to  $0.72$  after sequential exclusion of individual studies, with all confidence intervals crossing the null value. Overall, these sensitivity analyses indicate that the pooled results were robust across all time intervals and were not disproportionately influenced by any single study.

Fig. 13 presents the results of the leave-one-out sensitivity analyses conducted to assess the robustness of the pooled effect estimates at different exposure time points. At 24 hours, sequential omission of individual studies resulted in pooled standardized mean differences (SMDs) ranging from  $-0.38$  to  $0.08$ . In all omission scenarios, the corresponding 95% confidence intervals crossed the null value, indicating that exclusion of any single study did not produce a statistically significant change in the overall effect estimate. At 48 hours, the leave-one-out analysis showed that pooled SMDs ranged from  $-6.92$  to  $-1.81$  following exclusion of individual studies. Although variability in effect size magnitude was observed, none of the recalculated pooled estimates reached statistical significance, and all confidence intervals included zero, suggesting that the pooled results remained stable and were not driven by any individual study. Overall, these sensitivity analyses confirm the stability and robustness of the meta-analysis findings across the evaluated time points.

Fig. 14 presents the results of the leave-one-out sensitivity analyses performed to assess the robustness of the pooled effect estimates at different exposure time points. At 24 hours, sequential exclusion of individual studies resulted in pooled standardized mean differences (SMDs) ranging from  $-0.83$  to  $-0.22$ . In all omission scenarios, the corresponding 95% confidence intervals crossed the null value, indicating that exclusion of any single study did not lead to a statistically significant change in the overall effect estimate. At 48 hours, leave-one-out analysis demonstrated that pooled SMDs ranged from  $-1.49$  to  $-0.36$  following exclusion of individual studies. Although omission of one study resulted in a statistically significant pooled estimate, the direction of the effect remained

consistent across all exclusion scenarios, suggesting that the overall findings were not disproportionately driven by a single study. Overall, these sensitivity analyses indicate that the pooled results were generally stable across the evaluated time points, supporting the robustness of the meta-analysis findings.

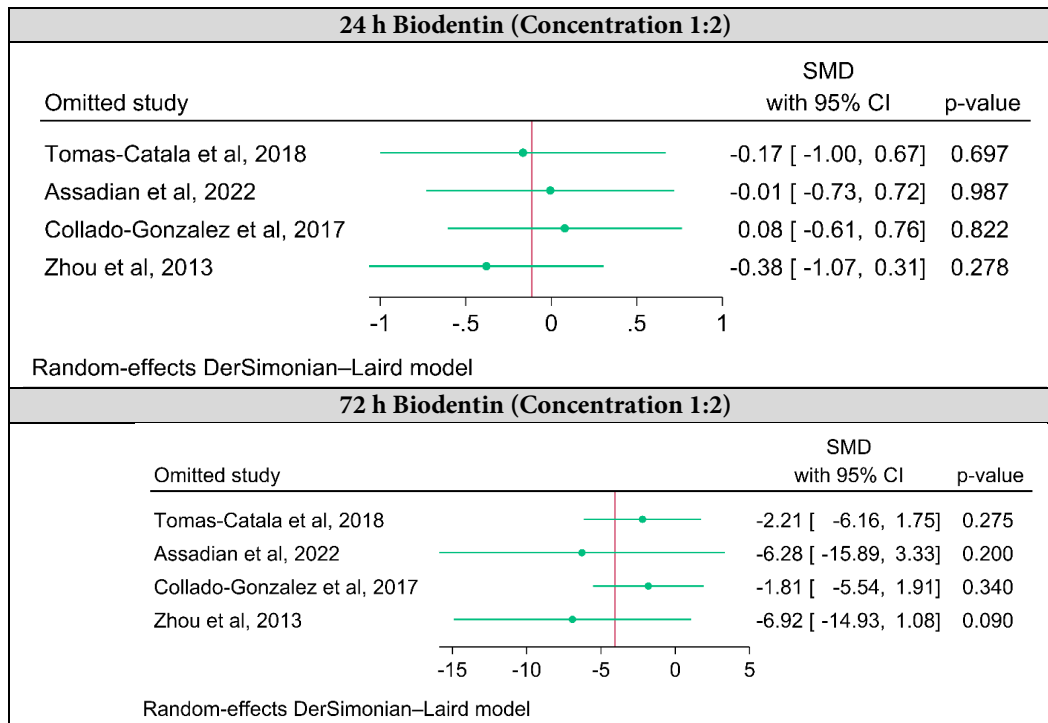
#### Publication bias

Assessment of publication bias using funnel plots and Egger's regression test was not performed, as the number of studies included in each group analysis was fewer than ten, which limits the reliability of these methods[72].

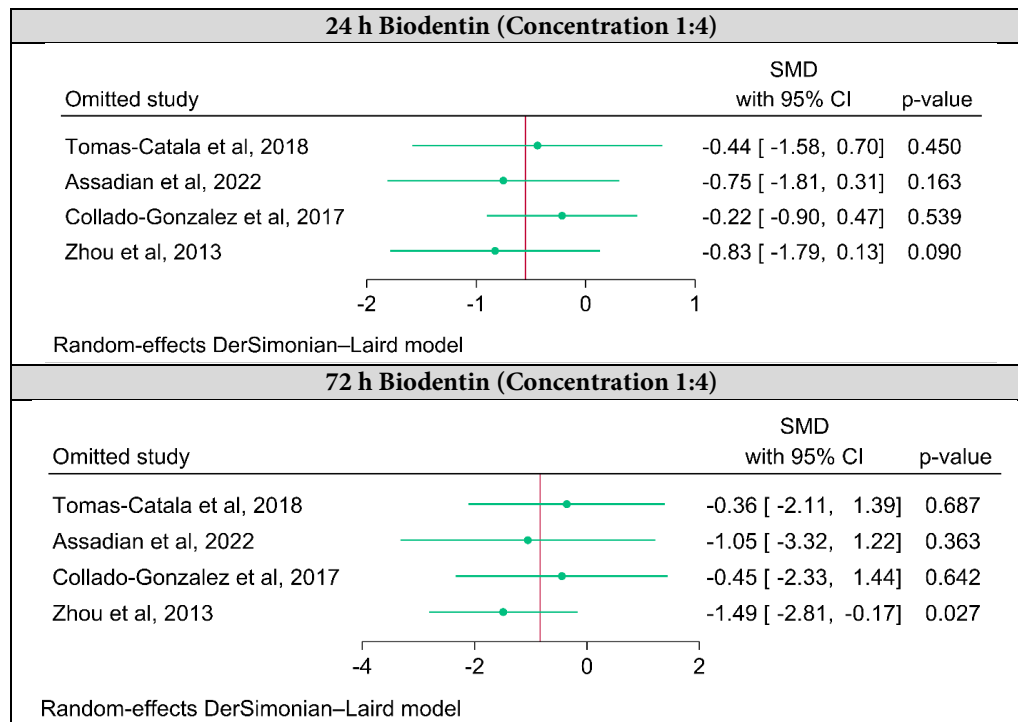
## Discussion

The sealer employed in root treatment has the potential to interact closely with extracellular fluids, eliciting various reactions around the root. An ideal sealer must possess several crucial properties, including biocompatibility and an adequate sealing margin. These characteristics facilitate the healing process of damaged and inflamed tissues (16). Given that no single material encompasses all the properties above, numerous sealing materials and techniques have undergone testing over the years, yielding varying degrees of success (15). The fact is shown that when exposed to human dental pulp cells, calcium silicate hydraulic cement exhibits heightened bioactivity. However, caution is warranted in the clinical application of these new materials, as even minor changes in their composition can significantly impact their clinical performance [73]. This study aimed to systematically review and investigate the cytotoxic effects of three types of calcium silicate-based cements: MTA, Biodentin, and CEM Cement. The results of the qualitative review, assessed using the ESA checklist, indicate no significant difference in cytotoxicity among these three substances.

The meta-analysis conducted in this study revealed that undiluted Mineral Trioxide Aggregate (MTA) and Biodentine at a 1:4 dilution were associated with a statistically significant reduction in cell viability at 48 hours after exposure. In contrast, no significant cytotoxic effects were observed for MTA at a 1:2 dilution at any evaluated time point. This observed difference may stem from the specific chemical compositions of these cements, warranting further research for a comprehensive understanding of the phenomenon. Furthermore, the distinct biological behavior of each cement, such as that of CEM Cement which is characterized by its unique calcium silicate-based composition setting via a hydration reaction and releasing calcium hydroxide, fosters bioactivity through the formation of hydroxyapatite. This mechanism underpins its well-d



**Figure 13.** Leave-one-out sensitivity analyses illustrating the influence of individual studies on the pooled standardized mean difference (SMD) estimates under the random-effects DerSimonian–Laird model. Each plot represents the pooled effect size recalculated after sequential omission of a single study. The consistency of results across omission scenarios indicates that the overall findings were robust and not disproportionately influenced by any individual study (95% CI).



**Figure 14.** Leave-one-out sensitivity analyses illustrating the influence of individual studies on the pooled standardized mean difference (SMD) estimates under the random-effects DerSimonian–Laird model. Each plot shows the recalculated pooled effect size following sequential omission of one study. The consistency of effect estimates across omission scenarios indicates that the overall results were robust and not driven by any individual study (95% CI).

ocumented osteogenic and cementogenic potential, contributing to its consistent biocompatibility and regenerative outcomes as evidenced in primary studies [12]. The meta-analysis outcomes, comparing the impact of these three substances on cell viability in terms of dosage and different time points, indicate no significant differences in their performance. Overall, the meta-analysis outcomes indicate no significant differences in cytotoxicity among the three materials under most experimental conditions, although transient, time-dependent effects were observed at specific time points.

In 2020, Pedano et al. conducted a systematic review and meta-analysis to explore the cytotoxicity and bioactivity of pulp coating agents. The study exposed human dental pulp cells of primary origin to various pulp coating agents, utilizing both *in vitro* and *in vivo* studies. It is noteworthy that the present review focuses exclusively on *in vitro* studies. Pedano's qualitative analysis of laboratory data revealed that resin-free hydraulic calcium silicate cement exhibited enhanced cell viability and bioactivity in human dental pulp cells compared to resin-based calcium silicate cement, glass ionomers, and calcium hydroxide cement. The excellent biocompatibility of Calcium Hydroxide powder (CH) and Pro-Root MTA, as demonstrated *in vitro* and *in vivo*, is largely consistent with the current findings; however, differences in study design, exposure conditions, and outcome assessment may account for variations observed in time-dependent cellular responses [73].

During the setting process of calcium silicate-based cement, calcium silicate hydrate is continuously formed, accompanied by the precipitation of calcium carbonate phosphate. Simultaneously, the release of calcium ions can lead to toxic inflammatory reactions [74]. However, it is crucial to note that releasing calcium ions from silicate cement is vital for the survival of mesenchymal stem cells (MSCs) [60]. These ions possess signaling capabilities, pivotal in regulating cell functions. Moreover, calcium ions influence the migration of various cell types, including mesenchymal stem cells (MSCs), bone marrow-derived mesenchymal stem cells (BMSCs), and tumoral cells [75, 76].

Calcium silicate-based cement, including MTA cement, Biodentine, and Cem cement, exhibits variations in cell viability percentages at different time points in this study, potentially attributed to differing releases of calcium ions. Saberi et al.'s 2016 study highlighted that the dissimilar releases of calcium ions in CEM and MTA cement could contribute to variations in cell viability percentages over time. MTA's chemical composition, distinct from CEM cement, changes with increasing bismuth and silica release but decreasing calcium ions. Bismuth oxide, released from MTA, has been documented

not to promote cell growth or proliferation [77]. Calcium ions, crucial for cellular activities, play a pivotal role in the biocompatibility of these biomaterials.

Further studies evaluating this aspect can aid in selecting appropriate biomaterials for root restorative treatments. Additionally, the elevated pH of the biomaterials is noteworthy, as it causes increased toxicity and influences cell survival in the early stages [78]. The gradual stabilization of MTA pH over time has been suggested as a potential factor contributing to improved cytocompatibility in later stages, rather than indicating a direct or significant increase in cell viability [45]. Early cellular responses within the first 48 hours, including alterations in cell density and substrate adherence, may influence subsequent reductions in cell viability, rather than indicating sustained cell proliferation. This finding could be a key factor contributing to the significant differences observed after 48 hours among these biomaterials [44]. Additionally, the choice of solvent in different studies, as demonstrated in previous research on MTA, can influence the trend of results, with physical properties improving when the material is in contact with PBS solvent [79].

A study conducted by De Deus et al. in 2005 evaluated the cytotoxic effects of two brands of mineral trioxide aggregate (MTA) (Pro-Root MTA and MTA Angelus) as well as Portland cement (PC) on the human endothelial cell line ECV304. They used the MTT method and found no statistically significant difference in the cytotoxicity of any test materials. Both brands of MTA and PC initially exhibited a similar cytotoxic effect, which gradually decreased over time, allowing the cell culture to recover. This phenomenon can be attributed to the high pH of MTA in the initial hours, which aligns with the findings of the present study [80].

A study conducted by Asgari and Ehsani in 2009 revealed that when the pulp tissue of the human third molar tooth is exposed to CEM cement, it forms a calcified barrier within the tooth, effectively encapsulating itself in the tooth's internal environment and achieving tissue regeneration. In other words, it demonstrated the ability of CEM cement to promote tissue regeneration [81]. Similarly, Khedmat et al. 2013 found that Biodentine and ProRoot MTA exhibited similar biocompatibility. However, Biosealer and CEM cement were significantly more cytotoxic to monocyte cells than ProRoot MTA and Biodentine after 48 hours of incubation. This difference in cytotoxicity could be attributed to the specific type of cells and ingredients used in the sealer formulations [4], which aligns with the results observed in the present study.

The study conducted by Zhou et al. in 2013 concluded that there was no significant difference in cell viability between

Biodentine and MTA throughout the entire experimental period, with a statistical significance level of  $P < 0.05$ . When human gingival fibroblasts were in contact with Biodentine and MTA for 24 hours, they attached to the surface of the materials and spread across the surface of the materials. Furthermore, the number of fibroblasts increased after 3 and 7 days of cultivation, indicating a favorable biocompatible response to both Biodentine and MTA [6]. The exclusion of 72-hour data from Zhou et al.'s (2013) study resulted in significant alterations to the sensitivity test outcomes. This discrepancy may be attributed to their use of flow cytometry methodology, which differs fundamentally from the MTT assay employed in other comparable studies.

Two studies conducted by Saberi and colleagues in 2016 yielded important findings regarding the cytotoxicity of various biomaterials on different cell types [44, 45]. Here are the key results:

On stem cells of the human apical papilla (SCAP):

At 24, 48, and 168 hours, the cytotoxicity of four substances (CEM, OCP, Biodentine, and MTA) did not differ significantly from that of the control group.

A two-by-two comparison revealed that the cytotoxicity of MTA and CEM differed significantly at 168 hours, with CEM showing lower cytotoxicity than MTA.

At 48 hours, the cytotoxicity of OCP and MTA was significantly different, with OCP demonstrating better biocompatibility than MTA.

CEM, OCP, Biodentine, and MTA exhibited acceptable biocompatibility when exposed to SCAP stem cells. CEM had the lowest cytotoxicity over time.

On human gingival fibroblasts (HGFs):

At 24 and 48 hours, the cytotoxicity of the four biomaterials (MTA, CEM, Biodentine, and OCP) did not differ significantly from that of the control group.

However, at 168 hours, a significant difference was observed between MTA and Biodentine compared to the control group. MTA had a p-value of less than 0.05, while Biodentine had a p-value of less than 0.01.

In summary, the cytotoxicity of MTA, CEM, Biodentine, and OCP against HGFs was similar to that of the control group at 24 and 48 hours. Over time, both MTA and Biodentine demonstrated less cytotoxicity compared to the other materials.

In 2020, Omidi and colleagues conducted a study demonstrating that MTA, CEM, and Biodentine biomaterials, even when used in different dilutions, did not exhibit cytotoxic effects, highlighting their biocompatibility [43]. Additionally, the results of studies conducted by Assadian et al. in 2022 indicated that optimal cell proliferation was observed when

evaluating the effects of three hydraulic calcium silicate cements on the odontoblastic differentiation of human dental pulp stem cells. Notably, this favorable cell response was observed across different concentrations and time points, emphasizing the positive impact of these materials on odontoblastic differentiation [64]. The results of the present study indicate that, although no significant differences in cytotoxicity were observed under most experimental conditions, material concentration and exposure duration may influence cellular responses at specific time points, particularly at 48 hours of exposure. This research suggests that within the parameters studied, the tested materials, including MTA, CEM, and Biodentine, exhibit consistent biocompatibility and do not exhibit substantial cytotoxic effects on the cells under investigation. These findings are valuable for understanding the safety and biocompatibility of these dental biomaterials across various conditions of use.

In 2017, Thomas-Catala and colleagues conducted a study assessing the compatibility of newly introduced pulp capping materials namely, MTA Repair HP, NeoMTA Plus, and Biodentine with human dental pulp stem cells (hDPSCs). Their findings indicated a favorable level of cell compatibility for all three materials, with Biodentine exhibiting higher rates of time-dependent proliferation [49]. The research suggested that over time, tissue damage improves, and cell proliferation reaches its peak within the initial 48 hours before stabilizing, which aligns with the current investigation's outcomes. In summary, the collective evidence suggests that these three biomaterials generally exhibit comparable biocompatibility; however, their biological effects may vary depending on concentration and exposure duration.

However, one of the important limitations of the present systematic review and meta-analysis lies in the insufficient number of eligible studies within certain subgroups—particularly those defined by specific combinations of material concentration, exposure duration, and cell type. This limitation may influence the internal validity and statistical power of subgroup analyses. This issue confirms this heterogeneity, according to the meta-analysis data expressed as the I<sup>2</sup> coefficient. As such, caution should be exercised when interpreting the findings from these smaller subgroups.

To strengthen the scientific foundation and clinical relevance of these results, future investigations should aim to fill these gaps by designing targeted, high-quality studies that focus on underrepresented variables, such as long-term exposure durations, diluted forms of biomaterials, and a broader range of human cell types. Additionally, in vivo animal models will be

essential as an intermediary step to bridge *in vitro* findings with eventual human clinical applications, offering a more holistic view of material biocompatibility in complex biological environments.

## Conclusion

This systematic review and meta-analysis demonstrated that the three evaluated CSCs (MTA, Biodentine, and CEM) generally exhibit favorable biocompatibility profiles and do not induce significant cytotoxic effects under *in vitro* conditions. Across multiple studies and time points, these biomaterials generally demonstrated acceptable biocompatibility with no substantial cytotoxic effects, although transient, time- and concentration-dependent cellular responses were observed.

Nevertheless, it is crucial to emphasize that these conclusions are derived exclusively from *ex vivo* (*in vitro*) studies. While *in vitro* models provide foundational insight into cytotoxic responses, they cannot fully replicate the complex biological interactions occurring *in vivo*. Therefore, before these findings can be confidently translated into routine clinical practice, further validation through animal studies and well-designed clinical trials is essential. Such investigations will help elucidate long-term biological responses, material-tissue interactions, and potential host-specific factors that may influence outcomes in real-world dental procedures.

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## Conflict of interest

None.

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## Authors' contributions

Conceptualization: SSPC/MH/EK, Methodology: AR/EK/MM, Formal analysis and investigation: SSPC/AR/EK Writing-original draft preparation: SSPC, Writing-review and editing: SSPC/MH/EK, Supervision: MH/EK. All authors read and approved the final manuscript.

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