

*Systematic Review*

# Comparative Biological Properties of Resin-Modified Calcium Silicate Cements on Dental Stem Cells: A Systematic Review of In Vitro Studies

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## Abstract

**Objectives:** This systematic review aimed to qualitatively synthesize the available in vitro evidence on the cytocompatibility and bioactivity of resin-modified calcium silicate cements in contact with human dental stem cells and cells from other sources. **Materials and Methods:** An electronic search was performed in five databases. In vitro studies evaluating the biological properties of resin-modified calcium silicate cements were included. Cytocompatibility outcomes comprised cell viability, cytotoxicity, migration, proliferation, adhesion, and apoptosis. Bioactivity outcomes comprised mineralization potential and the expression of osteo/odontogenic differentiation markers. A qualitative synthesis of the methodology and results of the selected studies was performed, together with an assessment of methodological and reporting quality using a modified CONSORT checklist for in vitro studies on dental materials. **Results:** Thirteen articles were included in the qualitative synthesis. Overall, the evaluated resin-modified calcium silicate cements showed reduced cytocompatibility compared with control groups, particularly regarding cell viability and migration. However, bioactivity findings were generally favorable, with several materials maintaining mineralization potential and the expression of markers associated with osteo/odontogenic differentiation. **Conclusions:** Resin-modified calcium silicate cements showed formulation-, concentration-, and time-dependent biological responses. Although some dual-cure materials retained mineralization potential and osteo/odontogenic activity, resin-containing formulations generally exhibited lower cytocompatibility than hydraulic calcium silicate cements. Favorable bioactivity should therefore not be interpreted as evidence of overall biological suitability, particularly when direct interaction with pulp tissue is expected. **Clinical Relevance:** Resin-modified calcium silicate cements should be used cautiously in vital pulp therapy, especially when direct interaction with pulp cells is expected.



Academic Editor: Corina  
Marilena Cristache

Received: 25 June 2026

Revised: 17 July 2026

Accepted: 22 July 2026

Published: 24 July 2026

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**Keywords:** resin; calcium silicate; cell culture; cytocompatibility; bioactivity; systematic review

## 1. Introduction

Vital pulp therapy (VPT) is a biologically based approach aimed at preserving the integrity and function of the dentin–pulp complex after deep caries, traumatic pulp exposure, or iatrogenic exposure during cavity preparation [1]. Its success depends on maintaining the survival, recruitment, and odontogenic differentiation potential of dental pulp stem cells (DPSCs), which may contribute to the formation of odontoblast-like cells and tertiary dentin. Therefore, materials used for pulp capping or pulpotomy should not only provide

an adequate seal and favorable handling but also support a biocompatible and bioactive cellular environment [2–6].

Hydraulic calcium silicate cements (CSCs) emerge as ideal candidates for such a purpose. The first CSC specifically developed for endodontic applications was mineral trioxide aggregate, initially introduced as a powder–liquid material for root-end filling and subsequently extended to perforation repair, apexification, pulp capping and pulpotomy. Conventional MTA formulations were principally composed of tricalcium and dicalcium silicates with a radiopacifier and were manually mixed with water before placement. Their hydration generated calcium silicate hydrate and calcium hydroxide, which are responsible for alkalinization, calcium-ion release and the formation of an apatite-like interfacial layer. Later powder–liquid formulations modified the calcium silicate composition, particle size, radiopacifier, liquid vehicle and setting accelerators to improve handling, setting time, discoloration potential and mechanical properties. Paste–paste, light-curable and dual-curable experimental formulations were subsequently introduced to further simplify placement and provide command or accelerated setting. However, the addition of hydrophobic monomers, polymerization initiators or other resin components can alter cement hydration, porosity, water uptake, calcium release and the availability of reactive calcium silicate phases. Consequently, the biological behavior of a calcium silicate material cannot be inferred solely from the presence of calcium silicate, but must be interpreted in relation to the complete formulation and its setting mechanism [7–9].

Most recently, ready-to-use premixed CSCs have been developed, particularly as root canal sealers and endodontic repair materials. These formulations generally contain calcium silicate particles dispersed in a non-aqueous or water-miscible vehicle and rely on moisture from dentine or the surrounding environment to initiate hydration. Their chemical and biological behavior may differ from that of manually mixed powder–liquid cements because the vehicle, water availability, particle dispersion and material thickness influence setting, ion release, alkalinization and apatite-forming ability. Recent investigations of premixed calcium-silicate-containing sealers have reported material-dependent differences in calcium and silicon release, surface mineral deposition, cytocompatibility and osteogenic signaling [10].

In parallel, resin-modified calcium silicate cements were developed to overcome the drawbacks of conventional CSCs by combining the bioactive potential of CSCs with the simplified handling and rapid light- or dual-curing polymerization of resin-based materials [11,12].

Nevertheless, the incorporation of a resin matrix remains biologically controversial. The resin component of resin-modified calcium silicate cements varies according to the formulation. Commercial and experimental materials may contain dimethacrylate monomers such as bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), ethoxylated bisphenol A dimethacrylate, or other functional methacrylates, together with photoinitiators or chemical initiator systems. Bis-GMA provides a highly cross-linked polymer network but is relatively viscous, whereas lower-molecular-weight monomers such as TEGDMA and HEMA may act as diluents and facilitate material handling and polymerization. The type and proportion of these monomers, as well as the curing mechanism and degree of conversion, influence water sorption, hydrolytic stability and the amount of residual components released from the set material. Residual unpolymerized monomers may diffuse directly into pulp tissue or indirectly through dentinal tubules, potentially impairing cell viability, migration, and differentiation [13,14]. This concern is reflected in the position statements of major endodontic societies, which favor hydraulic CSCs over resin-containing materials for VPT [15,16].

At the cellular level, biocompatibility is commonly assessed as cytocompatibility through outcomes such as viability, proliferation, migration, adhesion, morphology, and apoptosis [17–20]. Bioactivity is usually evaluated through mineral deposition and the expression of osteo/odontogenic markers [21]. Given that VPT outcomes depend on the maintenance of viable and functionally responsive pulp cells, a synthesis of the available in vitro evidence is clinically relevant. Therefore, this systematic review aimed to qualitatively assess the cytocompatibility and bioactivity of resin-modified calcium silicate cements in contact with human dental stem cells or cells from other sources.

## 2. Materials and Methods

### 2.1. Protocol and Registration

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [22]. The review protocol was registered in OSF Registries after completion of the search strategy, and before the title and abstract screening/data extraction/manuscript preparation. No deviations from the registered eligibility criteria or planned outcomes were made after registration. The registration DOI is as follows: <https://doi.org/10.17605/OSF.IO/AVD4K>.

### 2.2. Eligibility Criteria and PICOS Framework

The eligibility criteria were defined according to the PICOS framework. Eligible studies were full-text in vitro investigations evaluating the cytocompatibility and/or bioactivity of one or more resin-modified calcium silicate cements in human or animal cells. No restriction was placed on the specific cell lineage, provided that the study evaluated a cellular response to an eligible resin-modified calcium silicate material. Accordingly, studies using primary cells, stem cells or established cell lines were considered eligible.

Cytocompatibility was defined as the assessment of cell adhesion, apoptosis, migration, proliferation, viability and/or cytotoxicity. Bioactivity was defined as the assessment of mineralized matrix formation, alkaline phosphatase activity and/or the expression of markers associated with osteogenic or odontogenic differentiation.

The PICOS framework was structured as follows: population, human or animal cells; intervention, direct or indirect exposure to one or more resin-modified calcium silicate cements; comparator, untreated cells, vehicle controls or another dental material; outcomes, cytocompatibility and/or cellular bioactivity outcomes; and study design, in vitro studies.

Studies were excluded when they: (1) evaluated calcium silicate materials without a resin component; (2) assessed resin-based materials without a calcium silicate component; (3) did not include a cellular model; (4) evaluated only physicochemical, mechanical, antimicrobial or clinical outcomes without reporting any of the predefined cytocompatibility or bioactivity outcomes; (5) were animal, ex vivo or clinical studies without an eligible in vitro cellular component; (6) were reviews, systematic reviews, narrative reviews, editorials, letters, commentaries, protocols or technical reports; (7) were conference abstracts or other publications for which sufficient full-text methodological and outcome data were unavailable; (8) were doctoral dissertations or other unpublished academic documents; (9) represented duplicate reports of the same experiment, in which case the most complete report was retained; or (10) did not provide sufficient information to determine eligibility or extract the relevant results after assessment of the full text.

### 2.3. Information Sources and Search Strategy

An advanced electronic search was performed in five databases: MEDLINE via PubMed, Scopus, Embase, Web of Science, and SciELO. Database search was last updated

on 21 April 2026 to include the most recent eligible studies. No language restrictions were applied.

The search strategy included the following terms: resin, “calcium silicate”, “in vitro”, cytocompatibility, biocompatibility, differentiation, adhesion, apoptosis, migration, viability, cytotoxicity, proliferation, mineralisation, and mineralization. These terms were organized into three advanced search fields and combined using the Boolean operators “AND” and “OR”. The complete search strategy for each database is shown in Table 1.

**Table 1.** Search strategy.

| Database           | Search Field                                                                                                                                                                                         | Records   |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| MEDLINE via PubMed | #1: resin AND “calcium silicate”                                                                                                                                                                     | 560       |
|                    | #2: “in vitro”                                                                                                                                                                                       | 1,928,568 |
|                    | #3: (cytocompatibility OR biocompatibility OR differentiation OR adhesion OR apoptosis OR migration OR viability OR cytotoxicity OR proliferation OR mineralisation OR mineralization)               | 4,758,825 |
|                    | #1 AND #2 AND #3                                                                                                                                                                                     | 132 *     |
| Scopus             | #1: TITLE-ABS-KEY (resin AND “calcium silicate”)                                                                                                                                                     | 847       |
|                    | #2: TITLE-ABS-KEY (“in vitro”)                                                                                                                                                                       | 2,855,967 |
|                    | #3: TITLE-ABS-KEY (cytocompatibility OR biocompatibility OR differentiation OR adhesion OR apoptosis OR migration OR viability OR cytotoxicity OR proliferation OR mineralisation OR mineralization) | 5,113,976 |
|                    | #1 AND #2 AND #3                                                                                                                                                                                     | 94 *      |
| Embase             | #1: Broad search (resin AND “calcium silicate”)                                                                                                                                                      | 583       |
|                    | #2: Broad search (“in vitro”)                                                                                                                                                                        | 8,817,847 |
|                    | #3: Broad search (cytocompatibility OR biocompatibility OR differentiation OR adhesion OR apoptosis OR migration OR viability OR cytotoxicity OR proliferation OR mineralisation OR mineralization)  | 4,419,078 |
|                    | #1 AND #2 AND #3                                                                                                                                                                                     | 92 *      |
| Web of Science     | #1: TS = (resin AND “calcium silicate”)                                                                                                                                                              | 455       |
|                    | #2: TS = (“in vitro”)                                                                                                                                                                                | 2,086,803 |
|                    | #3: TS = (cytocompatibility OR biocompatibility OR differentiation OR adhesion OR apoptosis OR migration OR viability OR cytotoxicity OR proliferation OR mineralisation OR mineralization)          | 3,693,257 |
|                    | #1 AND #2 AND #3                                                                                                                                                                                     | 74 *      |
| SciELO             | #1: resin AND “calcium silicate”                                                                                                                                                                     | 6         |
|                    | #2: “in vitro”                                                                                                                                                                                       | 14,831    |
|                    | #3: (cytocompatibility OR biocompatibility OR differentiation OR adhesion OR apoptosis OR migration OR viability OR cytotoxicity OR proliferation OR mineralisation OR mineralization)               | 36,334    |
|                    | #1 AND #2 AND #3                                                                                                                                                                                     | 0 *       |

\* Total results for each database after the combination of individual search strings. Study selection was performed directly from these results after duplicate record removal.

#### 2.4. Study Selection

All records retrieved from the database searches were exported to Mendeley software (Elsevier, Amsterdam, The Netherlands) version 1.19.8 for duplicate removal. After duplicates were removed, titles and abstracts were screened according to the predefined eligibility criteria. The remaining records were assessed in full text to confirm their inclu-

sion in the qualitative synthesis. Study selection was independently performed by two examiners (S.B. and J.L.S.). In the event of doubt, a third examiner was consulted (L.F.).

### 2.5. Data Extraction

The bibliometric variables extracted from each included study were author and year of publication. The methodological variables extracted were the tested materials, cytocompatibility and/or bioactivity assays, and follow-up periods. For the synthesis of results, the studies were classified according to the statistically significant findings reported for each assay, the time points at which these findings were observed, and the reported level of statistical significance. Data extraction was independently performed by two examiners (S.B. and J.L.S.). In the event of doubt, a third examiner was consulted (L.F.).

### 2.6. Methodological and Reporting Quality Assessment

The methodological and reporting quality of the included studies was independently assessed by two reviewers using the modified CONSORT checklist proposed by Faggion for preclinical in vitro studies of dental materials [23]. The checklist comprises 14 items addressing the structured summary, scientific rationale, objectives or hypotheses, description of the intervention, definition and timing of outcome measures, sample-size determination, randomization and allocation procedures, blinding, statistical analysis, precision of estimates, study limitations, funding and protocol availability.

Each item was classified as fulfilled, not fulfilled or not applicable. Applicability was considered separately for each study according to its experimental design. Disagreements were resolved through discussion and, when necessary, consultation with a third reviewer. A descriptive percentage of fulfilled applicable items was calculated for each study.

In addition to the global percentage, the principal potential sources of methodological error were examined at item level, particularly sample-size determination, random allocation of experimental units, blinding of investigators or outcome assessors, standardization and reproducibility of the experimental procedures, completeness of outcome reporting, statistical analysis and reporting of precision estimates.

Items were considered not applicable only when the corresponding procedure could not reasonably be implemented within the reported experimental design; lack of reporting was otherwise recorded as non-fulfillment.

## 3. Results

### 3.1. Study Selection

The electronic search identified 392 records: 132 from MEDLINE via PubMed, 94 from Scopus, 92 from Embase, and 74 from Web of Science. No records were retrieved from SciELO. After duplicate removal, 182 records were screened by title and abstract. Of these, 169 were excluded because they did not meet the eligibility criteria. Thirteen full-text articles were assessed for eligibility, and all met the inclusion criteria. Therefore, 13 studies were included in the qualitative synthesis (Figure 1).

### 3.2. Characteristics of the Included Studies

The resin-modified calcium silicate cements evaluated in the included studies were TheraCal LC, TheraCal PT, TheraBase Ca, and an experimental light-curable MTA-based cement. The composition, manufacturer, and frequency of assessment of each material are shown in Table 2.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources

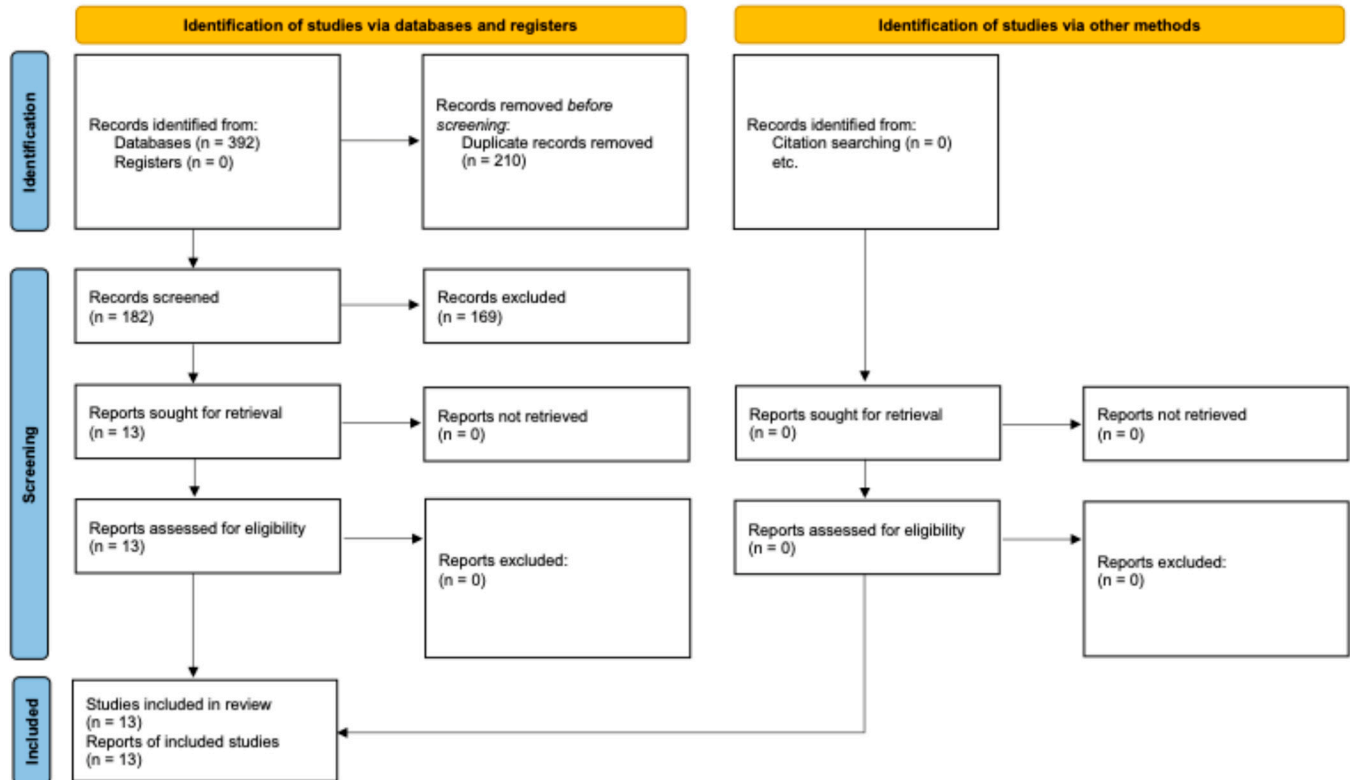


Figure 1. Study selection flow diagram based on the PRISMA 2020 statement [22].

Table 2. Composition of the resin-modified calcium silicate cements evaluated in the included studies.

| Material     | Composition                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Manufacturer, City, Country                                           | Number of Studies |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|-------------------|
| TheraCal LC  | Portland cement (30–50%), Bis-GMA (5–10%), barium zirconate (1–5%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | BISCO, Inc., Schaumburg, IL, USA                                      | 8                 |
| TheraCal PT  | Base: SG-Mix cement (50–75%), Bis-GMA (5–10%), barium zirconate (1–5%). Catalyst: Bis-GMA (10–30%), triethylene glycol dimethacrylate (10–30%), barium zirconate (5–10%), ytterbium fluoride (1–5%), benzoyl peroxide (<1%)                                                                                                                                                                                                                                                                                                                                      | BISCO, Inc., Schaumburg, IL, USA                                      | 7                 |
| TheraBase Ca | Base: barium ytterbium glass (30–50%), ethoxylated bisphenol A dimethacrylate (10–30%), Portland cement (10–30%), ytterbium fluoride (1–5%), sodium bromobenzenesulfinate dihydrate (1–5%), Bis-GMA (1–5%), titanium dioxide (<1%), acetyl-2-thiourea (<1%). Catalyst: glass filler (50–75%), 10-methacryloyloxydecyl dihydrogen phosphate (10–30%), triethylene glycol dimethacrylate (10–30%), diphenyl sulfone dimethacrylate (5–10%), silicon dioxide (1–5%), tert-butyl peroxybenzoate (1–5%), aluminum oxide (<1%), 2,6-di-tert-butyl-4-methylphenol (<1%) | BISCO, Inc., Schaumburg, IL, USA                                      | 2                 |
| LC-MTA       | Powder: dicalcium silicate, tricalcium silicate, tricalcium aluminate, barium sulfate, calcium sulfate, calcium chloride. Liquid resin: HEMA, TEGDMA, CQ, EDMAB                                                                                                                                                                                                                                                                                                                                                                                                  | Gandolfi MG and Prati C, University of Bologna patent, Bologna, Italy | 1                 |

The methodological characteristics of the included studies are shown in Table 3. Human dental pulp stem cells were used in 8 of the 13 included studies (61.5%), making them the predominant cell model. Stem cells from human exfoliated deciduous teeth were

used in 2 studies (15.4%), while SAOS-2 cells and Balb/3T3 fibroblasts were each used in one study (7.7%). One study (7.7%) did not clearly report the cell type.

**Table 3.** Methodological characteristics of the included studies.

| Study                               | Materials       | Cells                      | Cytocompatibility Assays                                                                                            | Bioactivity Assays                                                  |
|-------------------------------------|-----------------|----------------------------|---------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|
| Gandolfi et al., 2011 [12]          | LC-MTA          | SAOS-2 cells               | Alamar Blue (1, 3, 7 d); Neutral Red (2 h)                                                                          | NR                                                                  |
| Collado-González et al., 2017 [24]  | ThLC            | SHEDs                      | MTT (24, 48, 72 h); Annexin V-FITC/7-AAD (72 h); wound-healing assay (24, 48 h); SEM (3 d)                          | ARS (7, 14, 21 d)                                                   |
| Arias-Moliz et al., 2017 [25]       | ThLC            | NR                         | MTT (3 d)                                                                                                           | ALP (3 d)                                                           |
| Sanz et al., 2021 [26]              | ThPT, ThLC      | hDPSCs, 4th passage        | MTT (24, 48, 72 h); wound-healing assay (0, 24, 48, 72 h); Annexin V-FITC/7-AAD, SEM and phalloidin staining (72 h) | qRT-PCR (ALP, COL I, ON, DSPP, RUNX2; 14 d); ARS (21 d)             |
| Rodríguez-Lozano et al., 2021 [27]  | ThPT, ThLC      | hDPSCs, 2nd–6th passage    | MTT (24, 48, 72 h); wound-healing assay (0, 24, 48, 72 h); SEM, phalloidin staining and Annexin V-FITC/7-AAD (72 h) | qRT-PCR (ALP, COL1A1, ON, DSPP, RUNX2, BSP, GAPDH; 7 d); ARS (21 d) |
| Küden et al., 2022 [13]             | ThPT, ThLC      | hDPSCs                     | WST-1 (1, 3, 7 d)                                                                                                   | NR                                                                  |
| Kim et al., 2023 [28]               | ThPT, ThLC      | hDPSCs, 10th–12th passage  | CCK-8 (24, 48 h)                                                                                                    | qRT-PCR (COL I, OCN, OPN, GAPDH; 12, 48 h)                          |
| Novotná et al., 2024 [14]           | ThLC, ThPT      | Balb/3T3 mouse fibroblasts | MTT (24 h)                                                                                                          | NR                                                                  |
| Bakr et al., 2024 [29]              | ThPT            | hDPSCs, 4th passage        | MTT (1, 3, 7 d); wound-healing assay (2, 3 d)                                                                       | ARS, ALP and qRT-PCR (OPN, RUNX2, OCN, DSPP; 14 d)                  |
| Schuster et al., 2025 [30]          | ThLC            | hDPSCs                     | MTT, CyQUANT LDH and Live/Dead staining (48 h, 7, 14 d)                                                             | ALP activity and ARS (14 d)                                         |
| Gökdoğan et al., 2026 [31]          | ThPT            | SHEDs                      | MTT (24 h); wound-healing assay (0, 24 h)                                                                           | NR                                                                  |
| Rodríguez-Lozano et al., 2026a [32] | ThPT, ThB       | hDPSCs                     | MTT and wound-healing assay (24, 48, 72 h); phalloidin staining, SEM and Annexin V-FITC/7-AAD (72 h)                | qRT-PCR (ALP, DSPP, RUNX2, COL1A1; 14, 21 d); ARS (21 d)            |
| Rodríguez-Lozano et al., 2026b [11] | ThLC, ThPT, ThB | hDPSCs                     | MTT and wound-healing assay (24, 48, 72 h); phalloidin staining and SEM (72 h)                                      | qRT-PCR (ALP, RUNX2, COL1A1, DSPP, BSP, ON; 3, 7 d); ARS (21 d)     |

ThLC, TheraCal LC; ThPT, TheraCal PT; ThB, TheraBase Ca; NR, not reported; hDPSCs, human dental pulp stem cells; SHEDs, stem cells from human exfoliated deciduous teeth; h, hours; d, days; SEM, scanning electron microscopy; ALP, alkaline phosphatase; ARS, Alizarin Red S staining; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

All eligible studies evaluated material eluates or extracts for the cytocompatibility and bioactivity assays. Five studies also evaluated the direct contact of the materials with the tested cell populations via qualitative SEM analysis. The included studies assessed cytocompatibility and bioactivity using different biological assays. Cell viability was mainly evaluated using MTT (10 studies; 76.9%). Other viability or cytotoxicity assays included Alamar Blue, Neutral Red uptake, WST-1, CCK-8, LDH release, and live/dead staining. Cell

migration was mainly assessed using wound-healing assays (7 studies; 53.8%), apoptosis using Annexin V/7-AAD staining, and cell morphology using SEM or phalloidin staining.

Bioactivity was evaluated through mineralization and osteo/odontogenic differentiation assays. Alizarin Red S staining was used to assess mineralized nodule formation, alkaline phosphatase activity was used as an enzymatic differentiation marker, and qRT-PCR was used to assess the expression of osteo/odontogenic markers, including ALP, COL I, ON, DSPP, RUNX2, BSP, OCN, and OPN (6 studies; 46.2%).

Cytocompatibility assays were mainly performed at short-term intervals, commonly 24, 48, and 72 h, although some studies extended follow-up to 7 or 14 days. Bioactivity assays were performed over more heterogeneous periods. qRT-PCR was assessed from 12 h to 14 or 21 days, whereas Alizarin Red S staining was generally performed at 14 or 21 days.

### 3.3. Cytocompatibility Outcomes

The statistically significant cytocompatibility findings are summarized in Table 4.

**Table 4.** Cytocompatibility outcomes reported in the included studies.

| Study                              | Assay                    | Significant Findings                                                                                                                                                                                                                                                                                          |
|------------------------------------|--------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gandolfi et al., 2011 [12]         | Alamar Blue; Neutral Red | LC-MTA > Vitrebond (24 h; *).<br>LC-MTA < wTC-BA and ProRoot MTA (3 d; *).<br>LC-MTA < wTC-BA and ProRoot MTA; LC-MTA > Vitrebond (7 d; *).                                                                                                                                                                   |
| Collado-González et al., 2017 [24] | MTT; wound healing       | ThLC (1:1, 1:2) < negative control (24, 48, 72 h; ***).<br>ThLC (1:1, 1:2, 1:4) < negative control (24, 48 h; ***).                                                                                                                                                                                           |
| Arias-Moliz et al., 2017 [25]      | MTT                      | ThLC > Biodentine > LC, LC-TCS and LC-TCS-TO (1 d; **).                                                                                                                                                                                                                                                       |
| Sanz et al., 2021 [26]             | MTT; wound healing       | ThLC (1:1, 1:2, 1:4) < negative control (24, 48, 72 h; ***).<br>ThPT (1:1, 1:2) < negative control (24, 48, 72 h; ***); ThPT (1:4) < negative control (72 h; *).<br>ThLC (1:1, 1:2, 1:4) < negative control in wound-healing assay (24, 48, 72 h; ***).                                                       |
| Rodríguez-Lozano et al., 2021 [27] | MTT; wound healing       | ThLC (1:1, 1:2, 1:4) < negative control (24, 48, 72 h; ***).<br>ThPT (1:1, 1:2) < negative control (24, 48 h; ***); ThPT (1:1, 1:2, 1:4) < negative control (72 h; ***).<br>ThLC (1:1, 1:2) < negative control (24, 48, 72 h; ***); ThPT (1:1) < negative control (24, 48, 72 h; ***) in wound-healing assay. |
| Küden et al., 2022 [13]            | WST-1                    | ThLC, ThPT and MTA < negative control (1 d; ***).<br>MTA > ThLC and ThPT (1 d; **, 3 d; ****).<br>MTA, ThPT and ThLC < negative control (3 d; ***); ThPT and ThLC < negative control (3 and 7 d; ****).                                                                                                       |
| Kim et al., 2023 [28]              | CCK-8                    | ThLC and ThPT < negative control (48 h; *).                                                                                                                                                                                                                                                                   |
| Novotná et al., 2024 [14]          | MTT                      | 100% and 50% ThLC < Biodentine (24 h; ****); 25% ThLC < Biodentine (24 h; ***); 10% ThLC < Biodentine (24 h; *).<br>100% and 50% ThPT < Biodentine (24 h; ****); 25% ThPT < Biodentine (24 h; **).                                                                                                            |

Table 4. Cont.

| Study                               | Assay              | Significant Findings                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------------------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bakr et al., 2024 [29]              | MTT; wound healing | ThPT < Activa, Bio-C and NeoPutty (3, 7 d; *).<br>ThPT < Activa, Bio-C, NeoPutty and negative control in wound-healing assay (1, 2, 3 d; *).                                                                                                                                                                                                                                                                                                                |
| Schuster et al., 2025 [30]          | MTT; LDH           | 10%, 25%, 50% and 100% ThLC < ReViCal (1, 3 d; *).<br>10%, 25%, 50% and 100% ThLC < Pulprotec MTA (1, 3 d; **).<br>25%, 50% and 100% ThLC < ReViCal, Pulprotec MTA and MTA PulpCap in LDH assay (1, 3 d; ***).                                                                                                                                                                                                                                              |
| Gökdoğan et al., 2026 [31]          | MTT                | 5% and 10% ThPT > negative control (24 h; *).                                                                                                                                                                                                                                                                                                                                                                                                               |
| Rodríguez-Lozano et al., 2026a [32] | MTT; wound healing | ThPT (1:1) < negative control (24, 48, 72 h; ***).<br>ThB (1:1) < negative control (72 h; ***).<br>ThPT and ThB (1:1) < negative control in wound-healing assay (24, 48, 72 h; ***).                                                                                                                                                                                                                                                                        |
| Rodríguez-Lozano et al., 2026b [11] | MTT; wound healing | ThLC (1:1, 1:2, 1:4) < negative control (24, 48, 72 h; ***).<br>ThPT (1:1, 1:2) < negative control (24, 48, 72 h; ***).<br>ThB (1:1) < negative control (48, 72 h; ***); ThB (1:2) < negative control (72 h; **);<br>ThB (1:4) > negative control (24 h; ***).<br>ThLC (1:1, 1:2) < negative control (24, 48, 72 h; ***); ThPT (1:1) < negative control (24 h; **, 48, 72 h; ***); ThB (1:1) < negative control (24, 48, 72 h; ***) in wound-healing assay. |

ThLC, TheraCal LC; ThPT, TheraCal PT; ThB, TheraBase Ca; h, hours; d, days; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ .

For TheraCal LC, significantly reduced cell viability compared with untreated controls was reported at 24, 48, and/or 72 h in several MTT-based studies. Reduced cell migration compared with untreated controls was also reported in wound-healing assays. In comparative analyses, TheraCal LC showed significantly lower cell viability than Biodentine, ReViCal, Pulprotec MTA, or MTA PulpCap, depending on the assay and time point.

For TheraCal PT, significant reductions in cell viability compared with untreated controls were reported in several studies, mainly at higher extract concentrations and at 24, 48, and/or 72 h. WST-1 assays showed significantly lower viability for TheraCal PT than for MTA at 1 and 3 days. Other studies reported significantly lower viability or migration for TheraCal PT than for Activa, Bio-C, NeoPutty, or untreated controls. One study reported significantly higher viability for 5% and 10% TheraCal PT extracts compared with the untreated control at 24 h.

For TheraBase Ca, significant reductions in cell viability compared with untreated controls were reported for the 1:1 extract at 72 h and for the 1:1 and 1:2 extracts at 48 and/or 72 h. One study reported significantly higher cell viability for the 1:4 extract compared with the untreated control at 24 h. In wound-healing assays, TheraBase Ca showed significantly reduced migration compared with the untreated control at 24, 48, and 72 h.

For the experimental light-curable MTA-based cement, cell viability was significantly higher than that observed with Vitrebond at 24 h. At 3 and 7 days, the experimental material showed significantly lower viability than white tricalcium cement and ProRoot MTA.

### 3.4. Bioactivity Outcomes

The statistically significant qRT-PCR findings are summarized in Table 5, and the remaining bioactivity outcomes are summarized in Table 6.

**Table 5.** qRT-PCR outcomes reported in the included studies.

| Study                               | Significant Findings                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sanz et al., 2021 [26]              | ON and RUNX2: ThPT > negative control (14 d; ***).<br>ALP: ThPT < negative control (14 d; ***).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Rodríguez-Lozano et al., 2021 [27]  | DSPP and RUNX2: ThPT > negative control (7 d; ***).<br>ALP and COL I: ThPT < negative control (7 d; ***); BSP (7 d; *).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Kim et al., 2023 [28]               | OCN: ThPT, ThLC and Biodentine > negative control (12 h; *); Biodentine and ThLC > negative control and ThPT (48 h; *).<br>COL I: ThLC > ThPT, Biodentine and negative control (12 h; *); Biodentine > ThLC > negative control > ThPT (48 h; *).<br>OPN: ThPT and ThLC > negative control and Biodentine (12 h; *); ThPT > negative control, ThLC and Biodentine (48 h; *).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Bakr et al., 2024 [29]              | OPN, RUNX2 and OCN: ThPT < Activa and NeoPutty (14 d; *).<br>DSPP: ThPT < Activa (14 d; *).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Rodríguez-Lozano et al., 2026b [11] | DSPP: ThPT and ThB > negative control (3 d; *); ThPT > negative control (7 d; ***); ThPT and ThB < MTA Angelus (3 d; *); ThPT > ThB and MTA Angelus (7 d; ***).<br>RUNX2: ThPT > negative control (7 d; ***); ThPT and ThB < MTA Angelus (3 d; ***); ThPT > ThB and MTA Angelus (7 d; ***).<br>ON: ThB < negative control (3 d; **); ThPT > negative control (7 d; ***); ThPT < MTA Angelus (3 d; ***); ThPT > ThB (3 d; *); ThB < MTA Angelus (3 d; ***); ThPT and MTA Angelus > ThB (7 d; ***).<br>BSP: ThPT < negative control (3 d; *); ThPT > negative control (7 d; ***); ThPT < MTA Angelus (3 d; *); ThPT > ThB (7 d; ***); ThPT > MTA Angelus (7 d; **).<br>COL I: ThPT and ThB < MTA Angelus (3, 7 d; ***).<br>ALP: ThB < negative control (3, 7 d; *); ThPT > negative control (7 d; ***); ThPT and ThB < MTA Angelus (3 d; ***); ThPT and MTA Angelus > ThB (7 d; ***). |

ThLC, TheraCal LC; ThPT, TheraCal PT; ThB, TheraBase Ca; ALP, alkaline phosphatase; BSP, bone sialoprotein; COL I, type I collagen; DSPP, dentin sialophosphoprotein; OCN, osteocalcin; ON, osteonectin; OPN, osteopontin; RUNX2, runt-related transcription factor 2; h, hours; d, days; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

**Table 6.** Other bioactivity outcomes reported in the included studies.

| Study                               | Assay    | Significant Findings                                                                                                                                                            |
|-------------------------------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Arias-Moliz et al., 2017 [25]       | ALP      | LC-TCS-TO > LC, LC-TCS, ThLC and Biodentine (3 d; *).                                                                                                                           |
| Collado-González et al., 2017 [24]  | ARS      | ThLC < negative control (7, 21 d; **, 14 d; ***).                                                                                                                               |
| Sanz et al., 2021 [26]              | ARS      | ThLC < negative control (21 d; ***).<br>ThPT > negative and positive controls (21 d; ***).<br>Biodentine > ThPT (21 d; ***).                                                    |
| Rodríguez-Lozano et al., 2021 [27]  | ARS      | ThPT > negative control (21 d; ***).                                                                                                                                            |
| Bakr et al., 2024 [29]              | ARS; ALP | ARS: ThPT < Bio-C, Activa and NeoPutty (21 d; *).<br>ALP: ThPT > negative control (14 d; *); ThPT < Bio-C (14 d; *).                                                            |
| Rodríguez-Lozano et al., 2026a [32] | ARS      | ThPT > negative control (21 d; *).<br>ThB > negative control (21 d; ***).                                                                                                       |
| Rodríguez-Lozano et al., 2026b [11] | ARS      | ThLC < negative control (21 d; **).<br>ThPT and ThB > negative control (21 d; ***).<br>ThLC < ThPT, ThB and MTA Angelus (21 d; ***).<br>MTA Angelus > ThPT and ThB (21 d; ***). |

ThLC, TheraCal LC; ThPT, TheraCal PT; ThB, TheraBase Ca; ALP, alkaline phosphatase; ARS, Alizarin Red S staining; d, days; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

For TheraCal LC, significantly lower mineralized nodule formation than the untreated control was reported at 7, 14, and/or 21 days. In one study, alkaline phosphatase activity was significantly lower for TheraCal LC than for LC-TCS-TO at 3 days. qRT-PCR analyses showed significant differences in the expression of OCN, COL I, and OPN in comparisons involving TheraCal LC, TheraCal PT, Biodentine, and untreated controls.

For TheraCal PT, significantly higher mineralized nodule formation than the untreated control was reported at 21 days in several studies. qRT-PCR analyses showed significant changes in the expression of osteo/odontogenic markers, including ALP, COL I, ON, DSPP, RUNX2, BSP, OCN, and OPN, depending on the study and time point. In comparative analyses, Biodentine, Bio-C, Activa, NeoPutty, or MTA Angelus showed significantly higher mineralization or higher expression of selected differentiation markers than TheraCal PT in some studies.

For TheraBase Ca, significantly higher mineralized nodule formation than the untreated control was reported at 21 days. qRT-PCR analyses showed significant changes in the expression of ALP, DSPP, RUNX2, ON, BSP, and COL I. In direct comparisons, MTA Angelus showed significantly higher mineralization or higher expression of selected markers than TheraBase Ca at specific time points.

### 3.5. Methodological and Reporting Quality Assessment

The methodological quality assessment of the included studies is shown in Table 7. The overall percentage of item fulfillment ranged from 80% to 90%.

**Table 7.** Methodological and reporting quality assessment of the included studies.

| Study                        | Modified CONSORT Checklist Items for In Vitro Studies of Dental Materials ** |    |    |   |   |   |   |   |   |   |    |    |    |    | % |    |
|------------------------------|------------------------------------------------------------------------------|----|----|---|---|---|---|---|---|---|----|----|----|----|---|----|
|                              | 1                                                                            | 2a | 2b | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |   | 14 |
| Gandolfi et al. [12]         | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  |    |   | 80 |
| Collado-González et al. [24] | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Arias-Moliz et al. [25]      | *                                                                            | *  | *  | * |   | - | - | - | - | - | *  | *  | *  | *  |   | 80 |
| Sanz et al. [26]             | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Rodríguez-Lozano et al. [27] | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Küden et al. [13]            | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Kim et al. [28]              | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Novotná et al. [14]          | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Bakr et al. [29]             | *                                                                            | *  | *  | * |   | - | - | - | - | - | *  | *  | *  | *  |   | 80 |
| Schuster et al. [30]         | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Gökdoğan et al. [31]         | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Rodríguez-Lozano et al. [32] | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |
| Rodríguez-Lozano et al. [11] | *                                                                            | *  | *  | * | * | - | - | - | - | - | *  | *  | *  | *  |   | 90 |

\* Item fulfilled; - not applicable; %, percentage of item fulfillment per study. \*\* 1, structured abstract; 2a, scientific background and rationale; 2b, objectives and/or hypotheses; 3, detailed description of interventions; 4, definition and timing of outcome measures; 5, sample-size determination; 6, random-sequence generation; 7, allocation concealment; 8, implementation of allocation; 9, blinding; 10, statistical methods; 11, effect estimates and precision; 12, limitations and potential sources of bias or imprecision; 13, funding and role of funders; 14, accessibility of the full study protocol. Adapted from Faggion [23].

The methodological and reporting quality assessment is presented in Table 7. The percentage of fulfilled applicable items ranged from 80% to 90%. All 13 studies (100%) adequately reported the scientific background, objectives or hypotheses, experimental

interventions, and statistical methods. Outcome measures and their assessment were adequately described in 11 of 13 studies (84.6%). None of the included studies reported the availability of a prospectively accessible protocol.

A priori sample-size determination, random allocation of experimental units, allocation procedures, and blinding of investigators or outcome assessors could not be quantified from the present assessment because items 5–9 were classified as not applicable in all 13 studies. Effect estimates together with measures of precision were reported in all 13 studies (100%). Experimental interventions and procedures were described in sufficient detail to permit replication in all 13 studies (100%), although the definition and timing of outcome assessment were incompletely reported in 2 studies (15.4%).

## 4. Discussion

### 4.1. Primary Findings

This systematic review synthesized the available *in vitro* evidence on the cytocompatibility and bioactivity of resin-modified calcium silicate cements. The main finding was that these materials showed heterogeneous biological behavior depending on the formulation, cell model, extract concentration, and evaluation time point. Overall, resin-modified calcium silicate cements frequently showed reduced cytocompatibility compared with untreated controls or hydraulic calcium silicate cements, particularly regarding cell viability and migration. However, some formulations, especially dual-cure materials, also showed bioactive potential, as reflected by mineralized nodule formation and the expression of osteo/odontogenic markers.

### 4.2. Methodological Considerations

Human dental pulp stem cells were the most frequently used cell model among the included studies. This model is relevant for vital pulp therapy because these cells are involved in dentin–pulp repair and odontoblast-like differentiation [33]. Stem cells from human exfoliated deciduous teeth were also used in two studies and represent a dental stem cell population with odontogenic and mineralizing potential [34]. Other models included Balb/3T3 mouse fibroblasts and SAOS-2 cells, while one study did not specify the cell type used.

The included studies assessed cytocompatibility mainly through metabolic viability assays. MTT was the most frequently used test, followed by Alamar Blue, Neutral Red, WST-1, CCK-8, LDH release, and live/dead staining. However, MTT mainly reflects metabolic activity and should not be interpreted as a direct measurement of cell death without complementary assays [35]. Migration was evaluated using wound-healing assays, apoptosis using Annexin V/7-AAD staining, and cell morphology using scanning electron microscopy or phalloidin staining. Wound-healing assays are useful for evaluating collective cell migration, although their interpretation depends on experimental standardization and image analysis conditions [36]. Bioactivity was mainly assessed using Alizarin Red S staining, alkaline phosphatase activity, and qRT-PCR analysis of osteo/odontogenic markers. This methodological variability limited direct comparison between studies and supported the use of a qualitative synthesis.

### 4.3. Interpretation of Cytocompatibility Findings

TheraCal LC showed the most unfavorable cytocompatibility profile among the resin-modified calcium silicate cements evaluated. Several included studies reported reduced cell viability and impaired migration compared with untreated controls or hydraulic calcium silicate-based materials [11,13,14,24,26,27,30]. These findings are consistent with concerns regarding the biological effect of resin components in pulp-capping materials. Resin

monomers may remain unpolymerized after curing and may diffuse from the material, potentially affecting pulp cell viability [13,14,37]. In addition, dental polymer networks may undergo hydrolytic degradation in humid environments, which can contribute to the release of degradation by-products over time [38].

TheraCal PT was developed as a dual-cure resin-modified calcium silicate cement. In the included studies, it generally showed improved biological behavior compared with TheraCal LC, but reduced viability and migration were still reported in several comparisons, particularly at higher extract concentrations and longer exposure times [11,13,14,26–29,32]. Therefore, dual polymerization may improve the biological response compared with light-cured resin-modified materials, but it does not eliminate the cytotoxic potential associated with resin-containing formulations. This is consistent with the fact that even dual-cure resin systems may show incomplete conversion and residual monomer release [39].

TheraBase Ca was evaluated in fewer studies. The available evidence showed that it may present better short-term cytocompatibility than TheraCal LC and TheraCal PT at higher dilutions, although reduced viability and migration were still observed at higher concentrations [11,32]. Because only two included studies evaluated this material, these findings should be interpreted cautiously.

The experimental light-curable MTA-based cement showed higher cell viability than a resin-modified glass ionomer cement at early time points, but lower long-term cell viability than conventional MTA-based materials [12]. This supports the general observation that the addition of resin may improve handling but may also reduce the biological performance expected from hydraulic calcium silicate cements.

#### 4.4. Interpretation of Bioactivity Findings

The bioactivity findings were less uniform than the cytocompatibility findings. TheraCal LC generally showed limited mineralization potential, with lower Alizarin Red S staining values than untreated controls in several studies [11,24,26]. This suggests that although TheraCal LC contains calcium silicate components, the resin matrix may interfere with the biological response associated with hydraulic calcium silicate cements. This interpretation is consistent with previous evidence showing that resin-containing calcium silicate materials may present altered hydration behavior [40], which can compromise calcium ion release and bioactivity [41].

TheraCal PT showed a more favorable bioactive response than TheraCal LC. Several studies reported increased mineralization and upregulation of osteo/odontogenic markers, including RUNX2, DSPP, and ON, compared with untreated controls [11,26,27,32]. However, when TheraCal PT was compared with hydraulic calcium silicate cements or other calcium silicate-based materials, these comparators frequently showed equal or superior mineralization or marker expression [11,13,26,29,32]. Therefore, TheraCal PT appears to retain part of the bioactive potential of calcium silicate materials, although it does not consistently outperform hydraulic calcium silicate cements.

TheraBase Ca also showed bioactive potential, with increased mineralized nodule formation compared with untreated controls [11,32]. Nevertheless, its gene expression profile was not consistently superior to TheraCal PT or MTA Angelus. In direct comparisons, MTA Angelus showed higher mineralization or higher expression of some osteo/odontogenic markers at specific time points [11].

Overall, the results suggest that resin-modified calcium silicate cements may maintain some bioactive properties, particularly mineralization potential, but their cytocompatibility remains less predictable than that of hydraulic calcium silicate cements.

#### 4.5. Methodological Quality of the Included Studies

The included studies generally provided adequate descriptions of their objectives, experimental interventions, measured outcomes and statistical comparisons. Nevertheless, the item-level assessment identified several recurring methodological concerns. A priori sample-size determination was infrequently reported, limiting assessment of whether the experiments had sufficient statistical power. Random allocation of wells, specimens or experimental units and blinding of operators or outcome assessors were also rarely described. These procedures may be relevant even in cell-culture studies because plate position, specimen selection, microscopic-field selection and investigator-dependent image analysis can introduce systematic differences between groups.

The standardization of material preparation, extract generation, dilution ratios, cell density, passage number and exposure time was heterogeneous across studies. Although this variability does not necessarily indicate poor methodological conduct within individual experiments, it limits comparability and may contribute to differences in the reported biological responses. In addition, effect estimates were not always accompanied by measures of precision, and none of the studies provided access to a prospectively registered protocol.

The modified CONSORT checklist was used to evaluate methodological and reporting transparency rather than to assign a formal risk-of-bias category. Consequently, high item-fulfillment percentages should not be interpreted as definitive evidence of low systematic-error risk. The results instead identify reporting strengths and areas in which future *in vitro* studies should improve experimental planning, allocation procedures, assessor blinding, protocol standardization and complete statistical reporting.

#### 4.6. Limitations

This review has several methodological limitations. First, the search strategy was conducted in five electronic databases; however, relevant studies indexed in other sources may not have been identified. Second, all included studies were *in vitro* investigations. Therefore, the results cannot fully reproduce the clinical conditions of vital pulp therapy, where inflammation, bacterial contamination, dentin thickness, pulpal pressure, restorative sealing, and operative technique may influence pulp healing. Third, the included studies used different cell types, passage numbers, extract preparation methods, dilution ratios, biological assays, and follow-up periods. This heterogeneity prevented quantitative synthesis and limited direct comparison between studies. Also, the evidence for some materials, particularly TheraBase Ca, was limited because only a small number of studies evaluated them.

In addition, most included evidence was obtained using material extracts rather than direct cell–material contact. Extract models are useful for evaluating soluble leachates and permit standardized concentration-dependent comparisons, but they do not reproduce the interfacial conditions occurring when a pulp-capping material is placed directly over exposed pulp tissue. Conversely, direct-contact models may be affected by surface topography, specimen geometry, curing depth and local pH. The scarcity of direct-contact data therefore limits the clinical extrapolation of the present findings.

Regarding quality assessment, although the modified CONSORT checklist provides a structured appraisal of methodological and reporting transparency in *in vitro* dental-material studies, it is not a validated domain-based risk-of-bias tool. Consequently, the percentage of fulfilled items should not be interpreted as evidence of a low risk of systematic error. Furthermore, because items related to sample-size calculation, randomization, allocation, and blinding were classified as not applicable in the original assessment, firm conclusions regarding these potential sources of bias could not be drawn.

#### 4.7. Clinical Implications and Future Research

The contribution of this review is not to establish de novo that residual resin components may be harmful to pulp cells, but to determine whether different generations of resin-modified calcium silicate materials show a consistent cellular profile. The synthesis identified a clinically relevant dissociation between cytocompatibility and selected bioactivity outcomes: dual-cure materials sometimes promoted mineralization or differentiation markers despite simultaneously reducing viability or migration. Therefore, favorable expression of individual mineralization markers should not be interpreted as evidence of overall biological suitability.

From a clinical perspective, resin-modified calcium silicate cements should be used cautiously for vital pulp therapy. Their handling advantages and shorter setting or curing times may be clinically useful, but the available in vitro evidence suggests that these benefits may be accompanied by reduced cytocompatibility compared with hydraulic calcium silicate cements. Dual-cure formulations may show improved bioactivity compared with light-cured resin-modified materials, but the available evidence does not demonstrate that they are biologically superior to hydraulic calcium silicate cements.

Future studies should standardize cell culture protocols, extract preparation, dilution ratios, biological assays, and follow-up times to improve comparability. Further in vivo and clinical studies are also required to determine whether the in vitro differences observed between resin-modified and hydraulic calcium silicate cements translate into differences in pulp healing and long-term clinical outcomes.

## 5. Conclusions

Within the limitations of the available in vitro evidence, the biological response of resin-modified calcium silicate materials was formulation-, concentration- and time-dependent. Light-cured formulations, particularly TheraCal LC, consistently showed reduced cell viability, migration or mineralized matrix formation relative to untreated controls or hydraulic calcium silicate comparators. Some dual-cure formulations retained the capacity to promote mineralized nodule formation and the expression of selected osteo/odontogenic markers, but this bioactive response did not consistently coincide with favorable cytocompatibility. These findings indicate that the presence of calcium silicate phases alone does not confer biological equivalence to hydraulic calcium silicate cements.

**Author Contributions:** Conceptualization, S.B. and J.L.S.; investigation, S.B.; methodology, S.B. and J.L.S.; project administration, J.L.S.; resources, L.F., R.G. and J.G.; supervision, J.L.S.; validation, L.F., R.G. and J.G.; visualization, L.F., R.G. and J.G.; writing—original draft preparation, S.B.; writing—review and editing, J.L.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data from this manuscript are available upon reasonable request to the corresponding author.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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