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Scaffold-based Strategies for Direct Pulp Capping in Animal Models: A Systematic Review and Meta-analysis

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Abstract

Introduction: The dental pulp is a specialized connective tissue responsible for maintaining tooth vitality through a complex interplay of cellular, vascular, and immunological components. Despite the clinical use of conventional capping materials, their limited regenerative potential often results in pulp devitalization and compromised structural integrity. Recent advances in tissue engineering, particularly the application of three-dimensional scaffolds, have demonstrated promising outcomes in promoting dentin-pulp complex regeneration and biomimetic tissue repair. This systematic review and meta-analysis evaluated the efficacy of three-dimensional matrices, with or without bioactive materials, as direct pulp capping agents in animal models. Outcomes included cellularity, dentin thickness, dentin bridge formation, dystrophic calcification, inflammation control, and pulp organization compared to commercial materials. **Materials and Methods:** A comprehensive search was conducted in indexed databases and gray literature. A random-effects meta-analysis used standardized mean differences and the inverse variance method. Heterogeneity (I^2), publication bias (Egger's and Begg's tests), and risk of bias (SYRCLE's RoB tool) were assessed. Statistical analyses were conducted using RevMan ($P<0.05$). **Results:** Seventeen studies met the inclusion criteria, with 13 included in the meta-analysis. The risk of bias was predominantly low, yet the certainty of evidence was very low. Scaffolds significantly enhanced cellularity ($P=0.02$; $I^2=91\%$), dentin thickness ($P<0.00001$; $I^2=87\%$), and inflammation control ($P=0.03$; $I^2=20\%$) compared to controls. No significant differences were observed for dentin bridge formation ($P=0.30$; $I^2=63\%$), dystrophic calcification ($P=0.14$; $I^2=32\%$), or pulp organization ($P=0.10$; $I^2=0\%$). **Conclusion:** Three-dimensional scaffolds demonstrated potential in promoting cellular activity, dentin formation, and inflammation control. However, their impact on dentin bridge formation, pulp organization, and dystrophic calcification remains inconclusive. Further high-quality studies are required to validate their clinical applicability.

Keywords: Dentin; Direct Pulp Capping; Scaffold; Three-dimensional Matrices

Introduction

The dental pulp is composed of odontoblasts, immune system cells, endothelial cells, and extracellular matrix whose primary function is to maintain the vitality of the tooth [1]. Blood vessels supply the tissue with nutrients, and macrophages and T lymphocytes provide immunological protection against microorganisms. Pulp stem cells and odontoblasts play a vital role in regenerating dentin damaged by tooth wear or dental care, as a protective physical defense in removing exogenous stimuli by depositing tertiary dentin [2]. Thus, the dentin-pulp complex is based on the interrelationship between these tissues to protect the vitality of the pulp [1].

Pulp injuries can occur as a result of deep carious lesions, some types of restorative procedures [3], or dental trauma. Several treatment options are available, ranging from the less

invasive, including direct pulp capping, to the more invasive, such as pulpotomy and pulpectomy [4]. Cases of pulp capping with conventional materials such as MTA and Biodentine result in endodontic treatment rates of less than 15% between 1 and 2 years [5-7]. Despite root canal sealing and resolution of painful symptoms resulting in clinical success, these teeth become devitalized, fragile, and prone to postoperative fractures or other complications, including reinfection [8].

Tissue engineering is based on three pillars: cells, three-dimensional matrices (scaffolds), and growth factors. Three-dimensional matrices are described as materials that are responsible for promoting an interface between cells [9] and allow the transport of bioactive molecules and drugs, creating an environment that can mimic pulp tissue to allow dentinogenesis [10]. Another pillar that influences the cellular microenvironment and the behavior and differentiation of stem cells are biochemical signals [9]. These can be released into the tissue environment by growth factors, cytokines, and small molecules [9]. In pulp therapy, several studies have used growth factors [11], proteins [12, 13], mediators [14], and drugs [15, 16] associated with three-dimensional matrices.

Three-dimensional matrices stimulate an interaction between cells and biological systems to regenerate tissues, organs or their functions [9]. The positive results of this interaction can be seen in several studies on dentin bridge formation and pulp regeneration in its natural architecture [11, 12, 14, 16-18]. Therefore, the aim of this review was to explore the literature on the use of three-dimensional matrices, associated or not with bioactive materials, as pulp capping agents used under pulp injury in an animal model compared to treatments with commercial materials, critically evaluating evidence on the effectiveness in pulp tissue regeneration, dentin thickness formed on the pulplesion, dentin bridge, prevention of dystrophic calcification, inflammation control and organization of pulp tissue.

Materials and Methods

This systematic review/meta-analysis was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [19], ensuring transparency and methodological rigor. The review addressed the research question: "Which type of three-dimensional matrix shows the best regenerative behavior as a direct pulp capping agent in an animal model?" This question was formulated using the PICO framework: Population (P), animals; Intervention (I), pulp capping; Control (C), synthetic, organic, and hybrid matrices; Outcome (O), cellularity, dentin thickness, dentin bridging, dystrophic calcification, inflammation control, and pulp tissue organization.

Information sources

Data was obtained from seven main databases (PubMed, Scopus, Embase, Web of Science, COCHRANE, LIVIVO, LILACS) using an appropriate search strategy (Appendix) (Supplementary File 1), covering the period from January 2001 to March 2024. In addition, the 110 most relevant studies from Google Scholar were included as a source of gray literature. The search was conducted without restrictions on date or language to ensure comprehensive identification of potentially relevant studies. Duplicate references were excluded using EndNote X9 software (Thompson Reuters, New York, USA).

Inclusion criteria

The systematic review included animal model studies in which pulp exposure was performed and three-dimensional matrices were used as pulp capping agents. The scaffold had to be compared by histological analysis to a commercially used control group, looking for efficacy of cellularity, dentin thickness formed over the pulp lesion, dentin bridging, dystrophic calcification, inflammation control, and pulp tissue organization in which a quantitative analysis was carried out.

Exclusion criteria

Studies that met the following exclusion criteria were not included in this investigation: laboratory studies; systematic reviews; narrative reviews; clinical cases; randomized clinical studies; studies not written in English; and studies that did not use a commercially used material in the control group.

Study selection

The studies were selected in two stages. In the first phase, two reviewers (GBQ and ELCF) conducted a comprehensive search of all selected electronic databases from January 2001 to March 2024 and assessed the titles and abstracts of the studies using the Rayyan web application (Qatar Computing Research Institute, Doha, Qatar), an open-access tool recognized for optimizing the systematic review process [20]. Articles that did not meet the inclusion criteria were excluded in this phase. In the second phase, the full texts of the preliminarily selected articles were examined according to the established inclusion criteria. The final list of included references was reviewed by another team member (DAC). Any disagreements were resolved by consensus, and when necessary, two other members (DAC and PGBS) made the final decision. Statistical analyses were performed by PGBS.

Data extraction

The data collection process involved the thorough extraction of information from the selected studies by one reviewer (GBQ), followed by careful cross-checking of the data by a second reviewer

(DAC). After this stage, the two responsible authors (GBQ and DAC) conducted detailed discussions to resolve any disagreements and reach a consensus. If disagreements persisted, a third investigator (ELCF) was called in to make the final decision, thus ensuring the accuracy and reliability of the data analysis process.

The selected studies were carefully evaluated, and specific variables were recorded: animal species; pulp exposure; bioactive molecule; scaffold; commercial control; and origin of the three-dimensional matrix.

Risk of bias in individual studies

The risk of bias (RoB) of the included studies was assessed by an independent reviewer using the SYRCLE RoB tool, which is based on the Cochrane RoB tool and adapted for animal intervention studies [21]. This tool covers the following domains: (1) Was the allocation sequence adequately generated and applied? (2) Were the groups similar at baseline, or were they adjusted for confounders in the analysis? (3) Was the allocation to the different groups adequately concealed? (4) Were the animals randomly housed during the experiment? (5) Were the caregivers and/or investigators blinded to the intervention each animal received during the experiment? (6) Were animals selected at random for outcome assessment? (7) Was the outcome assessor blinded? (8) Were incomplete outcome data adequately addressed? (9) Are reports of the study free of selective outcome reporting? (10) Was the study apparently free of other problems that could result in a high risk of bias? The summary of RoB assessment was generated using RevMan software (Review Manager, version 5.3, Cochrane Collaboration, Copenhagen, Denmark).

Meta-analysis

Quantitative data were analyzed using a continuous meta-analysis (random effects) using standardized mean differences (SMDs) and inverse variance method, and *Cohen's d* was calculated. The combined relative risk was performed equally using random effects and inverse variance method I2 coefficient was calculated to estimate the heterogeneity between studies and subgroups, one-of-out analysis was performed to identify the weight of each study and Egger and Beggs tests were used to identify risk of bias publication. Subgroup analyses were performed based on the nature of the scaffold molecules. All analyses were performed using the RevMan software ($P<0.05$).

Quality of evidence

The quality of the evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach, which analyzes specific items based on the reliability of estimates of effect or association [22].

The GRADE profiler summarized the quality of the evidence using the GRADE pro-GDT software (http://gdt.guidelinedevelopment.org). Depending on the importance of certain aspects (study design, risk of bias, consistency, targeting, heterogeneity, precision, publication bias, and others reported by the studies included in the systematic review), the quality of the evidence could be reduced by one or two levels for each aspect.

Results

Study selection

Initially, 574 articles were obtained from the main databases. After removing duplicate articles, the titles and abstracts of 453 studies were evaluated, and 39 potentially relevant studies were selected for detailed reading and evaluation according to the inclusion criteria. Four studies were excluded because they were written in a non-English language, one study was excluded because its full version could not be found, nine did not address the central theme of the review, and eight studies did not have commercial control. Finally, 17 studies were considered eligible for this systematic review, with 13 studies included in the meta-analysis (Fig. 1).

Risk of bias

For the studies included in the meta-analysis and the systematic review itself (Fig. 2), the frequencies of low, moderate, and severe RoB were 54%, 35% and 10%, respectively. Assessments of individual items based on the MAStARI tool showed a high RoB for the allocation sequence and blinding of investigators. An uncertain RoB was attributed to the randomization of animal housing during the experiment, according to both meta-analyses and systematic reviews (Fig. 3).

Characteristics of the studies

Seventeen studies carried out between 2001 and 2024 using animal models with pulp exposure and three-dimensional matrices as capping agents were characterized. Six studies used dogs as models (Lee *et al.* [23]; Obeid *et al.* [24]; Li *et al.* [25]; Yun *et al.* [26]; Farzad-Mohajeri *et al.* [27]; Sedek *et al.* [28]), and eleven studies used rats (Goldberg *et al.* [29]; Sharma *et al.* [17]; Liu *et al.* [30]; Zhu *et al.* [18]; Okamoto *et al.* [31]; Guerrero-Gironés *et al.* [32]; Wang *et al.* [14]; Yan *et al.* [15]; Cunha *et al.* [12]; Xu *et al.* [33]; Han *et al.* [34]). Direct pulp capping was the common therapy, but there was variation in cavity formation and pulp exposure (Table 1).

The studies used different bioactive molecules, with scaffolds varying according to their origin. Seven studies used three-dimensional matrices of synthetic origin (Goldberg *et al.* [29]; Lee *et al.* [23]; Obeid *et al.* [24]; Zhu *et al.* [18]; Okamoto *et al.* [31]; Guerrero-Gironés *et al.* [32]; Yan *et al.* [15]), eight of

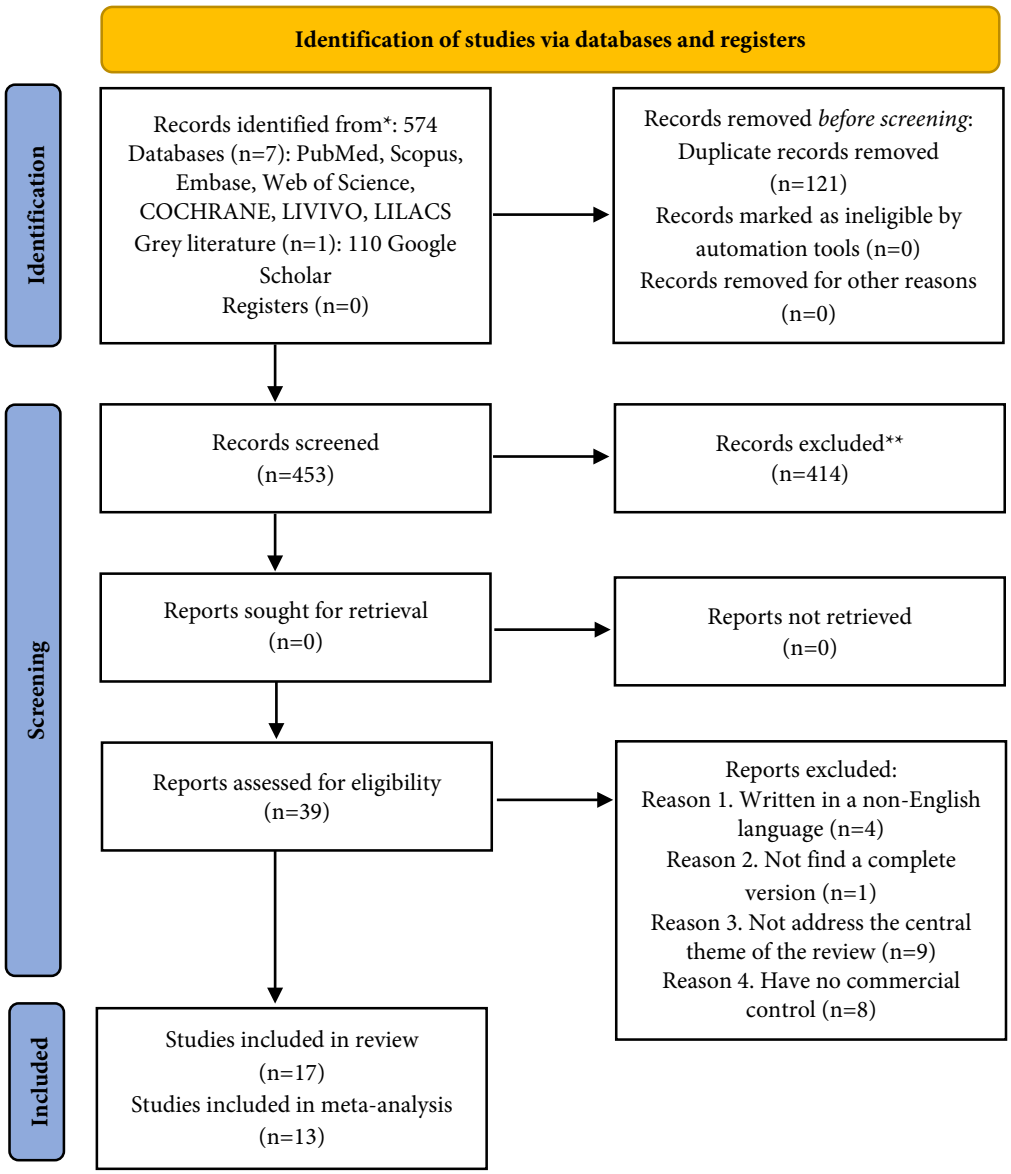

Figure 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

**Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers); **if automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation too*

organic origin (Li *et al.* [25]; Yun *et al.* [26]; Liu *et al.* [30]; Sharma *et al.* [17]; Wang et al. [14]; Cunha *et al.* [12]; Sedek *et al.* [28]; Xu *et al.* [33]), and five of hybrid origin (Guerrero-Gironés *et al.* [32]; Wang *et al.* [14] (Table 2).

Results of the meta-analysis

With regard to cellularity, the experimental groups obtained a standardized mean difference (SMD) of 1.45 (95% CI: 0.49 to 2.40) compared to the control groups, showing a positive effect of the experimental treatment. Heterogeneity between the studies was high, with I^2=91% (P<0.001), demonstrating significant variability in the results obtained. The general effect tests showed a Cohen's *d* of +3.79 [95% CI=0.57 to 7.02] (P=0.020). In the leave-one-out analysis, removing the data from each individual study significantly diluted the difference in favor of the experimental group (Fig. 4), and Egger's test (P=0.071) showed no significant risk of publication bias (Supplementary File 2).

With regard to dentin thickness, the experimental groups showed a significant increase compared to the control groups (P<0.001), with a standardized mean difference (SMD) of 3.47 (95% CI: 2.14 to 4.80). Heterogeneity between the studies was specific, with I^2=87% (P<0.001). There was no statistically

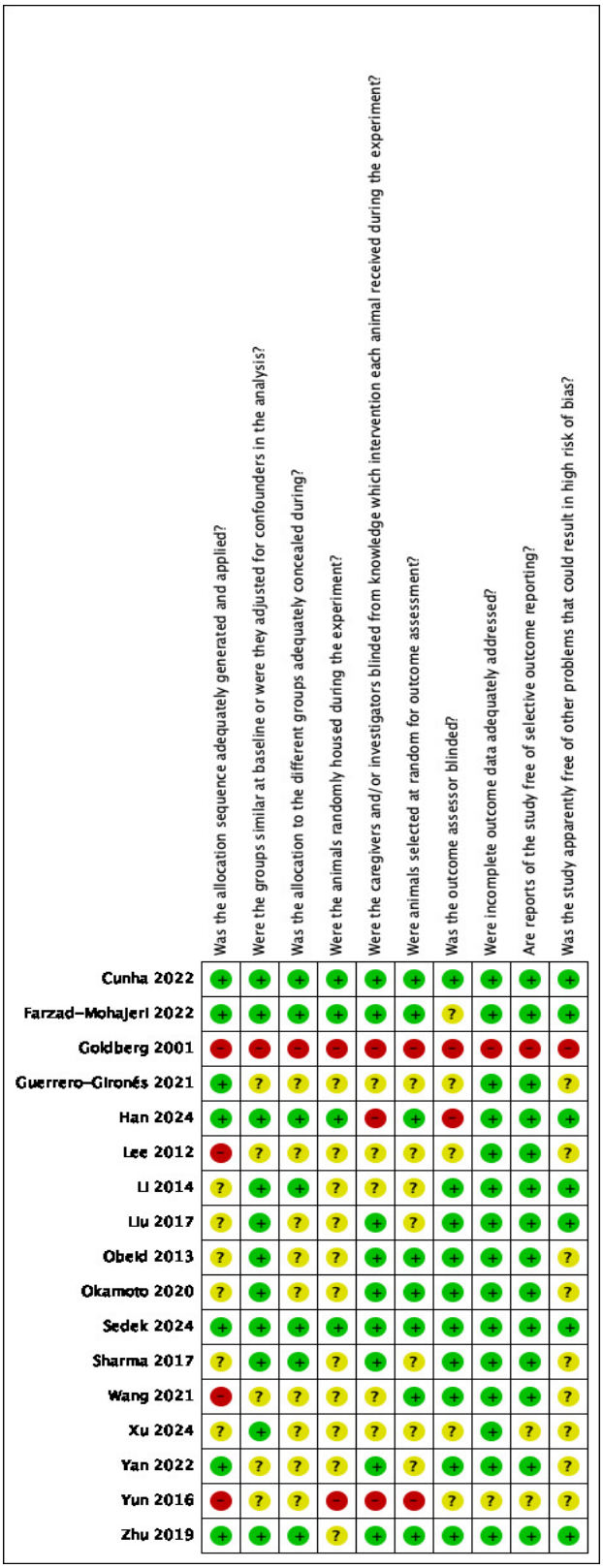

Figure 2. Risk of bias summary: review of the author's assessment of each RoB item for each study included. Symbol colors: green, low RoB; yellow, nuclear RoB; red, high RoB

significant difference between the subgroups of scaffolds enriched with organic and synthetic molecules (P=0.060). In the one-of-out analysis, removing the data from each study individually did not significantly dilute the difference in favor of the experimental group (Fig. 5), and Egger's test (P=0.001) showed a significant risk of publication bias (Supplementary File 3).

Regarding dentin bridging, the experimental groups showed no significant difference compared to the control group (P=0.030). The heterogeneity among the studies was moderate but significant (I^2=63%, P=0.005). There was no statistically significant difference between the subgroups of scaffolds enriched with organic and synthetic molecules (P=0.910). In the one-of-out analysis, the removal of any single dataset did not alter the difference in favor of the scaffold group (P>0.05) (Fig. 6). However, Begg's test (P=0.044) indicated a significant risk of publication bias (Supplementary File 4). These data suggest that the intervention did not produce a statistically significant effect on dentin bridge formation. Thus, the results indicate that, despite the variations observed, the experimental treatments do not offer an advantage over the control.

With regard to dystrophic calcification, the experimental groups showed no significant difference compared to the control group (P=0.140). The heterogeneity between the studies was not significant (P=0.230), the synthetic molecules subgroup (P=0.020), but not the organic molecules subgroup (P=1.00), benefited from the incorporation of molecules into the scaffold. In the leave-one-out analysis, removal of the data from Sharma et al. favored the experimental group (P=0.020) (Fig. 7). Begg's test (P=0.601) indicated no significant risk of publication bias (Supplementary File 5).

With regard to inflammation, the experimental groups showed a reduction of 0.44 (95% CI: 0.21 to 0.92) in the incidence of inflammation compared to the control groups. The heterogeneity between the studies was not significant (P=0.290). There was no statistically significant difference between the subgroups of scaffolds enriched with organic or synthetic molecules (P=0.410). In the one-of-out analysis, removing the data from Liu et al. and Zhu *et al.* [18] substantially reduced the difference in favor of the scaffold group (P>0.05) (Fig. 8). Begg's test (P=0.117) indicated no significant risk of publication bias (Supplementary File 6). Overall, these findings suggest that experimental treatments may effectively reduce inflammation incidence, indicating a potential advantage over controls.

With regard to tissue organization, the experimental groups showed no significant difference compared to the control (P=0.100), there was no heterogeneity between the studies (P=0.53), no proportion in the results (Fig. 9), and it was not possible to construct a funnel plot or perform the Beggs test because only two studies were included in this meta-analysis.

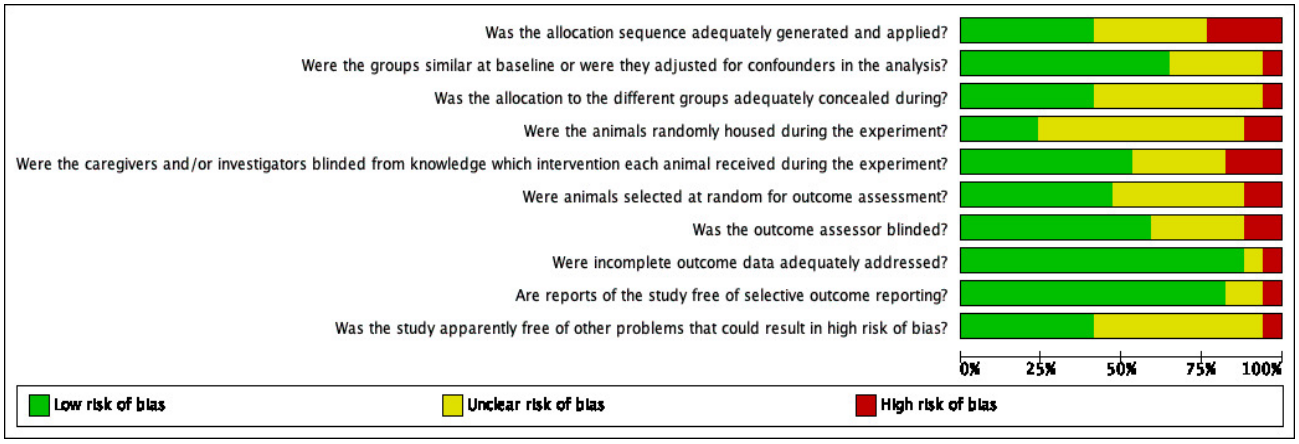

Figure 3. Risk of bias graph: review of the author's assessment of each RoB item, presented as porcentages across all included studies. Symbol colors: green, low RoB; yellow, nuclear RoB; red, high RoB

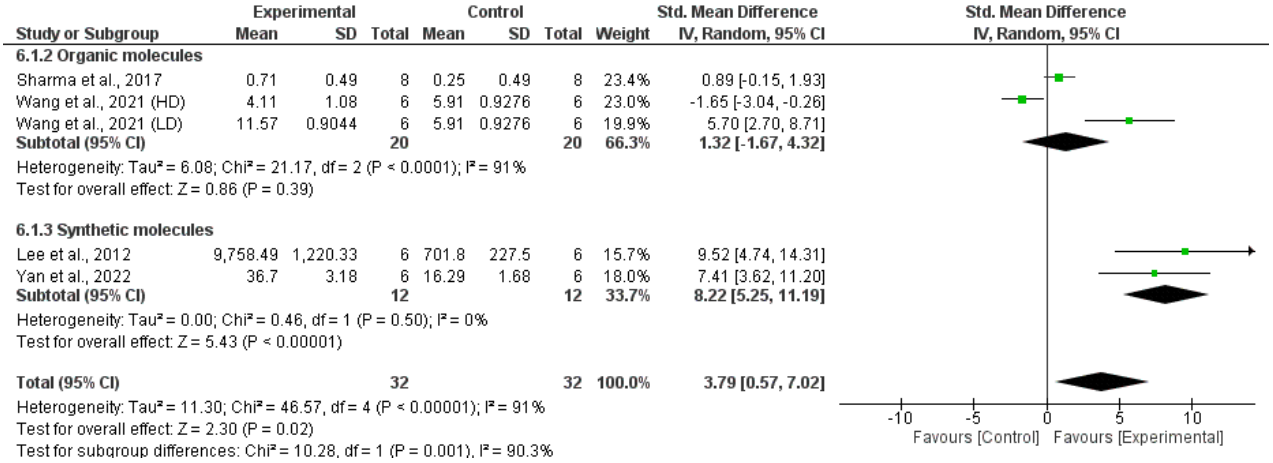

Figure 4. Forest plot of the cellularity

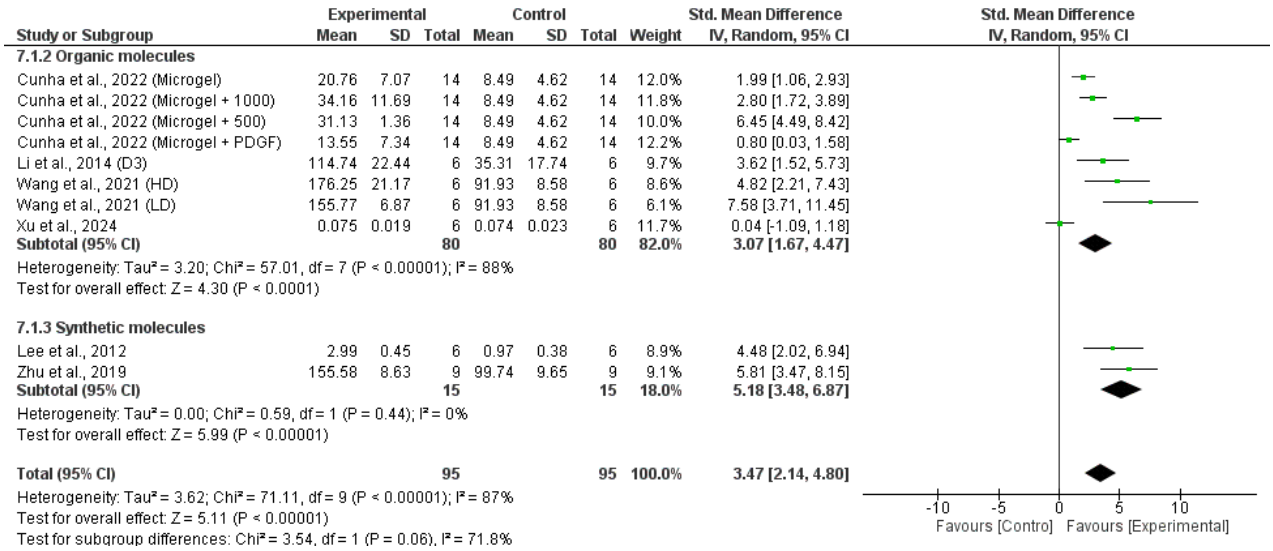

Figure 5. Forest plot of the dentin thickness

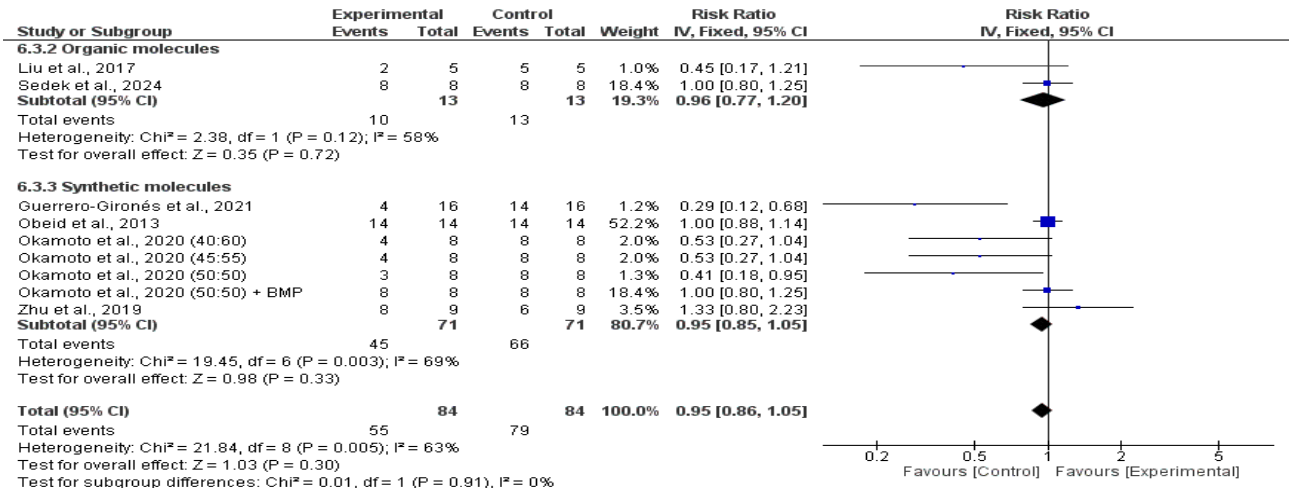

Figure 6. Forest plot of the dentin bridging

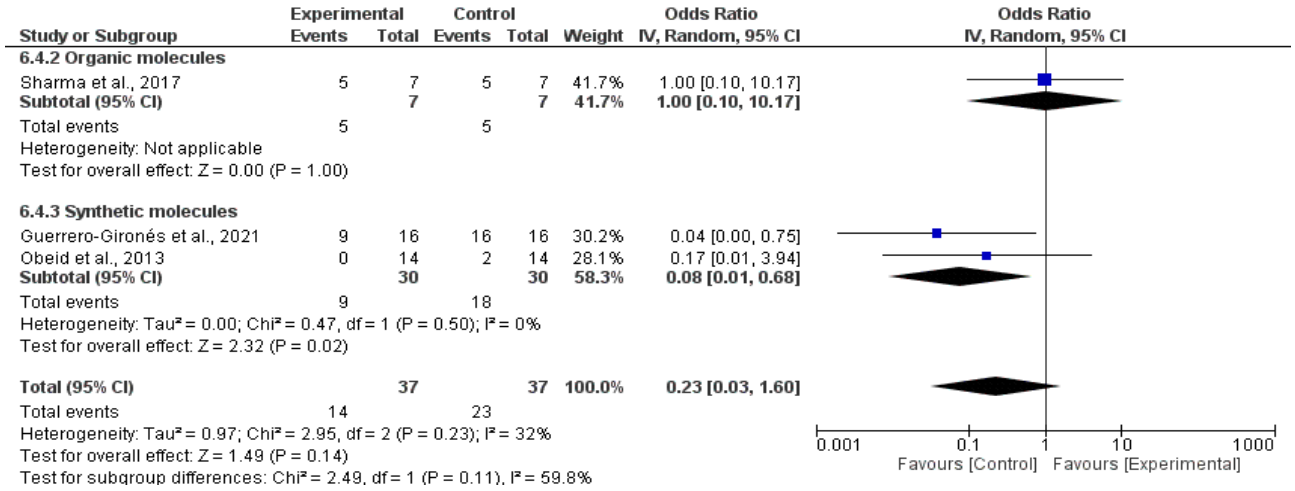

Figure 7. Forest plot of the dystrophic calcification

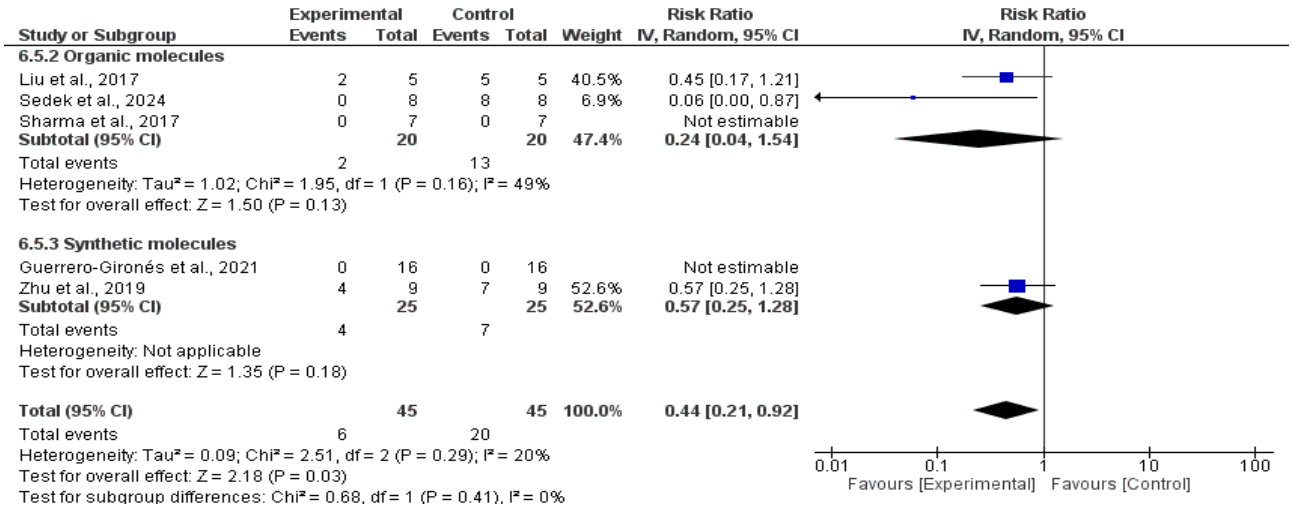

Figure 8. Forest plot of the inflammation

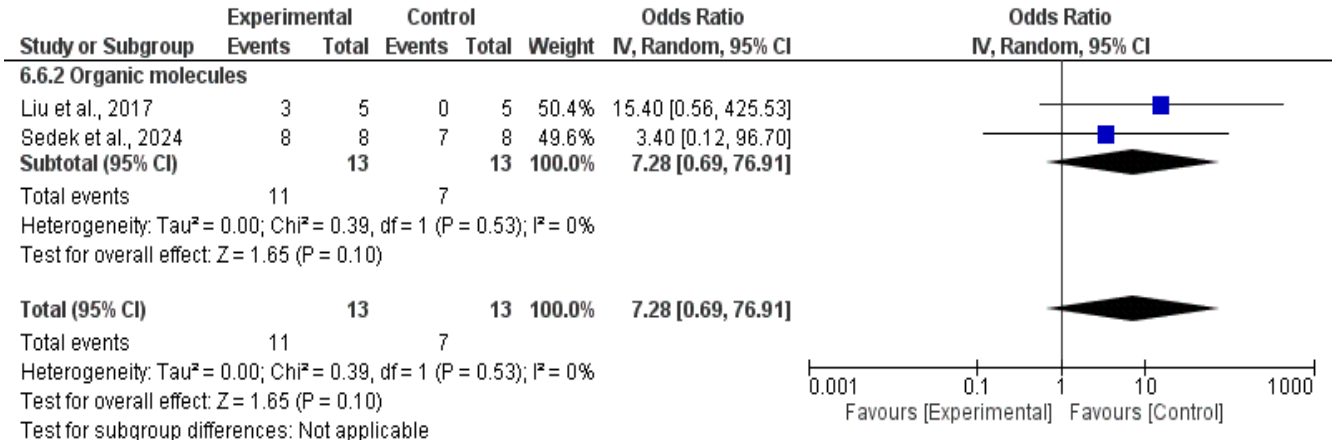

Figure 9. Forest plot of the tissue organization

Reliance on cumulative evidence–GRADE

According to the evaluation based on GRADE criteria, the certainty of evidence was considered very low in the assessment of cellularity, dentin thickness, dentin bridge, dystrophic calcification, inflammation, and pulp tissue organization. The evaluation of the evidence is described in detail in Table 3.

Discussion

In the present study, we observed that the experimental groups treated with scaffolds showed a significant increase in cellularity, dentin thickness, and a reduction in inflammatory findings when compared to the controls. However, dentin bridge formation, dystrophic calcification, and tissue organization showed no significant differences between the groups. Heterogeneity analysis and the risk of publication bias provided additional information on the robustness of these findings.

In the intricate relationship between dentin and pulp, the constituent elements of the dentin matrix play a crucial role in the sequence of biological events, with the potential to drive the multiplication of pulp progenitors/stem cells, allowing their cell populations to expand even before the differentiation process [35]. Previous studies by AlQahtani *et al.* [36] and Hu *et al.* [37] highlighted a significant increase in cellularity resulting from the proliferation of dental pulp-derived stem cells. This effect was observed with the use of scaffolds produced from decellularized dental pulp extracellular matrix, since they have the ability to mimic the native microenvironment that resembles natural pulp tissue, favoring tissue regeneration and adequate structural support. In addition, these structures contain bioactive molecules that play a crucial role in the recruitment of host cells, contributing significantly to the success of regenerative processes.

These results are in line with the findings of Lee *et al.* [23], Okamoto *et al.* [31], Sharma *et al.* [17] and Wang *et al.* [14], where the substantial impact on cellularity was evidenced by a Cohen's *d* of 3.79. However, the heterogeneity between the studies was high (I^2=91%), possibly due to methodological differences, such as type of scaffold, animal model, evaluation time and histological analysis methods. Sensitivity analysis indicated that removing data from individual studies significantly reduced the difference in favor of the experimental group, suggesting that some studies may have a disproportionate influence on the findings. However, Egger's test (*P*=0.071) indicated no significant risk of publication bias, giving the results greater reliability.

Studies highlight the importance of dentin thickness in promoting effective tissue regeneration, since dentin serves as a natural scaffold that supports dental pulp regeneration and the formation of new dentin tissue. The bioactivity of scaffolds can be maintained even in the presence of thick dentin, indicating that thicker dentin sections may allow for adequate diffusion and bioactivity of scaffolds used in endodontic applications [38]. Studies by Araújo *et al.* [39], and Wang *et al.* [40] highlight the potential of using scaffolds to not only provide support, but also actively promote dentin wall thickening during the regenerative process, and further support the notion that scaffold composition and design can enhance the regenerative process by promoting cell differentiation and increasing dentin thickness.

These findings are in line with the results found by Cunha *et al.* [12], Lee *et al.* [23], Li *et al.* [25], Wang *et al.* [14], Xu *et al.* [33] and Zhu *et al.* [18], which suggest that the use of three-dimensional matrices in direct pulp capping can contribute to thicker dentin neoformation, possibly due to the induction of a more intense cellular response and the deposition of mineralized matrix.

Table 1. Main study design

Author	Animal	Exposed pulp
Goldberg *et al.* [29]	Sprague-Dawley rats	-Cavity prepared in the mesio-cervical region of the right and left maxillary first molars, using round (0.6 mm) tungsten carbide drills -Punctual exposure of the pulp was carried out with a steel probe
Lee *et al.* [23]	Beagle dogs	-Cavity made with a #4 carbide bur in the central fossa of the upper second and third molars -Pulp exposure performed with a 1.5 mm diameter round drill.
Obeid *et al.* [24]	Dogs	-Cavity made with a #2 carbide bur on the buccal surface 2 mm above the gingival margin of the upper and lower canines and premolars -Pulp exposure was performed with a needle
Li *et al.* [25]	Beagle dogs	-Cavity made on the buccal surface of premolars and molars using a 0.5-mm diameter diamond drill (SS White) - Exposure of the pulp in the middle of the axial walls using a 0.5-mm diameter diamond drill (SS White)
Yun *et al.* [26]	Beagle dogs	- The cavity was a class V made in one third of the buccal cervical region of the canine teeth, punctually exposing the pulp by means of a pin
Liu *et al.* [30]	Wistars rats	-Cavity made with a ¼ carbide bur (Mani, Tochigi, Japan) in the mesial marginal crest of the first molars to open the pulp and ½ carbide bur to remove the pulp cap -Coronal pulp removed with small spoon
Sharma *et al.* [17]	Sprague-Dawley rats	-Cavity made with a 1/4 carbide bur in the central fossa of the upper first molars
Zhu *et al.* **[18]**	Wistars rats	-Cavity made using drills with a terminal diameter of 0.5 mm on the occlusal face of the maxillary first molars -Coronal pulp tissue was damaged using sterile K-file #40 endodontic files
Okamoto *et al.* [31]	Wistar rats	-Class I cavity was prepared on the occlusal surface of the maxillary first molars -Pulp exposure with a round steel drill No. 1 (Dentsply Sirona, Ballaigues, Switzerland)
Guerrero-Gironés *et al.* [32]	Sprague-Dawley rats	-Cavity made with a 0.8-mm diameter pyriform Tungsten carbide bur (Dentsply Sirona, York, PA, USA) in the maxillary first and second molars
Wang *et al.* **[14]**	Sprague-Dawley rats	-Cavity made with a ¼ carbide bur (0.5 mm diameter, SS White Burs, USA) in the central fossa of the maxillary first and second molars -Pulp exposure with an endodontic file (#20; Maillefer Dentsply, Switzerland)
Cunha *et al.* **[12]**	Wistars rats	-Cavity made with a 1/4 carbide bur in the central fossa of the maxillary first and second molars -Pulp exposure with an endodontic file (#15; Dentsply Maillefer, Ballaigues, Switzerland)
Yan *et al.* [26]	Sprague-Dawley rats	-Cavity made using 0.5 mm terminal diameter drills in maxillary first molars -Coronal pulp tissue was damaged using sterile K-file #40 endodontic files
Farzad-Mohajeri *et al.* [27]	Pedigree dogs	-Cavity made with a carbide burr on the vestibular side 1 mm above the gingival margin of the upper and lower premolars and molars -Pulp exposure was performed with a needle
Sedek *et al.* [28]	Dogs	-Class V cavities prepared in the cervical third, 0.5-1 mm above the gingival margin, using a sterile round carbide bur (Komet, Lemgo, Germany) -Pulp tissue exposed in the center of the cavity floor using an endodontic explorer (DG16, Dental USA Inc., McHenry, IL, USA)
Xu *et al.* [33]	Sprague Dawley rats	-Cavity made with a ¼ round carbide bur (diameter 0.5 mm; SS White Burs, Lakewood, NJ, USA) in the mesial half of the maxillary first molars -Pulp exposure performed with a sterile K#15 file
Han *et al.* **[34]**	Fischer Rats	-Cavity made in the central portion of the occlusal surface of the maxillary first molars with a 1/2 round bur (Kavo-Kerr, Orange, CA, USA) -Pulp exposure performed with a #25 sterile endodontic file (Dentsply Maillefer, Balligues, Switzerland)

Table 2. Characterization of the studies in terms of bioactive material, scaffold, positive control, and origin of the three-dimensional matrix

Organic			
Author	**Bioactive molecule**	**Scaffold**	**Positive control**
Li *et al.*, [25]	TGF-1 growth factor	Chitosan bilayer membrane	Radiopaque calcium hydroxide ($Ca(OH)_2$, Dycal, Dentsply Caulk, Milford, DE, USA)
Yun *et al.* [26]	-	Demineralized dentin paste	MTA (ProRoot, Dentsply, Tulsa Dental, Tulsa, Oklahoma, USA)
Liu *et al.* [30]	-	Demineralized bone matrix	Calcium hydroxide ($Ca(OH)_2$, Dycal, Dentsply International, York, PA, USA)
Sharma *et al.* [17]	None	Keratin (20% p/v)	Calcium hydroxide ($Ca(OH)_2$)
Wang *et al.* [14]	Notoginsenoside R1	Methacrylated gelatin (MA-gel) of 10%, 15% and 20% concentration loaded with HD: high drug load, LD: low drug load, ND: no drug load	Calcium hydroxide ($Ca(OH)_2$, Dycal, Dentsply, Germany)
Cunha *et al.* [12]	Dentin matrix proteins (500 mg/mL; 1000 mg/mL)	Methacrylated gelatin (gel-MA) in microgel form with 7% concentration	MTA (Angelus, Londrina, PR, Brazil)
Sedek *et al.* [28]	-	Injectable and light-curing gelatin-treated dentin matrix (LCG-TDM)	MTA
Xu *et al.* [33]	-	Chondroitin sulfate (CS) immobilized in type I collagen hydrogels (CS-0; CS-0.1)	MTA
Synthetic			
Goldberg *et al.* [29]	-	Bone sialoprotein Bone morphogenetic protein-7 N-acetyl-cysteine	Calcium hydroxide ($Ca(OH)_2$)
Lee *et al.* [23]	None	Polycaprolactone fiber mesh	MTA
Obeid *et al.* [24]	Autogenous stem cells derived from bone marrow	Hydroxyapatite tricalcium phosphate	MTA (ProRoot1; Dentsply Tulsa, Tulsa, OK)
Zhu *et al.* [18]	-	Silver-doped bioactive glass particles/ Injectable chitosan/β-glycerophosphate gel	MTA (Dentsply Endodontics, USA)
Okamoto *et al.* [31]	BMP-2 (bone morphogenetic protein-2)	Ploy (ε-caprolactone-co-d,l-lactide) (P(CL-co-DLLA)) (CL:DLLA); NPC (50:50/ 45:55/ 40:60)	MTA (WMTA; Dentsply Sirona, OK, Lot 108824)
Guerrero-Gironés *et al.* [32]	-	Mixture of 40% hydroxyapatite (Sigma-Aldrich, Darmstadt, Germany), 30% beta-tricalcium phosphate (Sigma-Aldrich, 49963, Darmstadt, Germany) and 30% collagen (Sigma-Aldrich, C4387 Darmstadt, Germany)	MTA (Dentsplay Maillefer, Ballaigues, Switzerland)
Yan *et al.* [26]	Aspirin	Membrane composed of three layers: Lower: aspirin Intermediate: aspirin/calcium phosphate/poly-L-lactic acid Top: poly (lactic-co-glycolic acid) (PLGA)	MTA (Dentsply, York, PA, USA)
Hybrid			
Farzad-Mohajeri *et al.* [27]	Autogenous stem cells derived from bone marrow	Collagen/hydroxyapatite hybrid structure	MTA (ProRoot MTA; Dentsply, York, USA)
Han *et al.* [34]	-	Gelatin methacrylate (GelMA) electrospun, incorporating beta-tricalcium phosphate (TCP) particles (GelMA+20% TCP)	MTA (Angelus, Londrina, Brazil)

Table 3. Grade

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Scaffolds	None or others	Relative (95% CI)	Absolute (95% CI)		
Cellularity												
5	observational study	not serious	serious	not serious	serious	none	32	32	-	SMD 3.79 SD higher (0.57 higher to 7.02 higher)	Very low	Critical
Dentin thickness												
10	observational study	not serious	not serious	not serious	serious	none	95	95	-	SMD 3.47 SD higher (2.14 higher to 4.8 higher)	Very low	Critical
Dentin bridge formation												
9	observational study	not serious	serious	not serious	serious	none	84	84	-	SMD 0.95 SD higher (0.86 higher to 1.05 higher)	Very low	Critical
Dystrophic calcification												
3	observational study	not serious	not serious	not serious	serious	none	37	37	-	SMD 0.23 SD higher (0.03 higher to 1.6 higher)	Very low	Critical
Inflammatory findings												
5	observational study	not serious	not serious	not serious	serious	none	45	45	-	SMD 0.44 SD higher (0.21 higher to 0.92 higher)	Very low	Critical
Organization of pulp tissue												
2	observational study	not serious	not serious	not serious	serious	none	13	13	-	SMD 7.28 SD higher (0.69 higher to 76.91 higher)	Very low	Critical

CI: Confidence interval; SMD: Standardised mean difference

However, heterogeneity between the studies was high (I^2=87%), indicating considerable variability in the findings. Sensitivity analysis showed that removing individual studies did not significantly alter the positive effect observed, reinforcing the robustness of the results. However, Egger's test (*P*=0.001) indicated a significant risk of publication bias, which suggests that the available studies may overestimate the impact of scaffolds on dentin thickness.

Studies investigating the effects of native and recombinant growth factors such as TGF-β, BMPs, FGFs, and IGFs in pulp capping situations *in vivo* in animal models [40, 41] have shown evidence of stimulation of hard tissue formation. However, there was heterogeneity in the responses, with some discrepancies in the effects of individual molecules, probably due to the complexity of local events, such as bacterial microleakage and inflammation [42]. The formation of fibrodentin followed by tubular dentin suggests that the induction of dentinogenic events may have been indirect but significant.

In the present study, there was no statistically significant difference in dentin bridge formation between the experimental and control groups (*P*=0.030), suggesting that the use of three-dimensional matrices in direct pulp capping did not provide a clear advantage in this variable. Heterogeneity between the studies was moderate but significant (I^2=63%), indicating considerable variation between the findings, possibly due to differences in the scaffold, time of evaluation, and animal model used. The sensitivity analysis showed that removing individual data did not significantly modify the difference between the groups (*P*>0.05), reinforcing the stability of the results. However, Begg's test (*P*=0.044) indicated a significant risk of publication bias, which can impact the reliability of the available evidence. Thus, although the experimental treatment showed good results in relation to dentin bridge formation, the findings suggest that the scaffolds do not provide a clear benefit in dentin bridge formation compared to the control, as reported by Elkady *et al.* [43] and Mangione *et al.* [44].

In addition to histological outcomes, scaffold degradation rates, mechanical properties, and immunogenicity may have influenced the

present findings. Scaffolds that degrade too rapidly may fail to provide sustained mechanical support and protection, potentially exposing the pulp to microbial infiltration and persistent inflammation [9]. Conversely, excessively slow degradation can hinder natural tissue remodeling and lead to the persistence of non-functional mineralized deposits [45]. Mechanical properties are equally critical: insufficient stiffness may result in scaffold collapse before tissue maturation, whereas excessive rigidity can impose undue stress on surrounding cells, impairing the formation of well-organized mineralized tissue [46]. Furthermore, even minimal immunogenic responses triggered by residual chemicals or bioactive components can alter the inflammatory microenvironment, influencing cellular differentiation and favoring irregular mineralization, including dystrophic calcification [45, 46].

Inflammation control improved (P=0.03), but pulp organization showed no significant difference (P=0.10), indicating that modulation of the inflammatory response by scaffolds although important for reducing tissue damage and clinical symptoms is not sufficient to restore the histological architecture of the dental pulp. Complete tissue regeneration requires not only the resolution of inflammation but also the coordinated activation of biological pathways that drive the differentiation of stem cells into functional odontoblasts, the organized deposition of extracellular matrix, and proper vascular and neural integration. Mild inflammation is a key initiator of healing, as it stimulates the recruitment and activation of reparative cells [47]; however, without adequate biochemical cues, such as cytokines and growth factors, repair may result in fibrotic and disorganized tissue rather than functional regeneration [48]. Achieving an optimal balance between inflammation and repair is therefore essential for restoring normal pulp tissue organization.

Several studies have indicated that there may be no significant difference in the formation of tissue organization when using scaffolds compared to MTA and calcium hydroxide, especially in bone and dental tissue applications. Xia *et al.* [49] demonstrated that porous calcium phosphate cement (CPC) could increase the formation of dentin-like mineralized tissue when used in conjunction with human dental pulp cells (hDPCs). Their findings suggested that CPC not only supported cell proliferation, but also promoted the expression of odontoblastic markers, indicating its potential as an effective support for dental tissue regeneration. This aligns with the properties of MTA, which is known for its biocompatibility and ability to stimulate the formation of reparative dentin, suggesting that CPC may function similarly to MTA in promoting tissue organization. Studies by Huan *et al.* [50] and Fu *et al.* [51] also suggest that scaffolds can achieve tissue organization comparable to that of traditional materials such as MTA and calcium hydroxide.

The results of the meta-analysis corroborate this result, since the experimental groups treated with scaffolds showed no statistically significant difference compared to the control groups (P=0.100), suggesting that the use of these three-dimensional matrices did not directly influence tissue organization. Furthermore, there was no heterogeneity between the studies (P=0.53), which indicates a certain consistency in the findings, but the inclusion of only two studies limits the strength of the evidence. The impossibility of constructing a funnel plot or carrying out Begg's test due to the small number of studies makes it impossible to carry out a robust assessment of publication bias.

The unusually high standardized mean difference (SMD) observed for pulp tissue organization appears to be primarily driven by Liu *et al.* [30], which had a small sample size (n=5 per group). Small cohorts can disproportionately inflate effect sizes due to reduced variability and greater influence of individual data points. While this study contributed substantially to the observed effect, its findings should be interpreted with caution, and larger, well-powered trials are needed to confirm these results within the broader context of the meta-analysis.

This study showed that the experimental groups had no statistically significant difference compared to the control in dystrophic calcification (P=0.140), with no evidence of significant heterogeneity between the studies (P=0.230). However, in the one-of-out analysis, the exclusion of data from Sharma *et al.* [17] favored the experimental group. In that study, it was observed that the implantation of keratin hydrogel resulted in the formation of mineralized deposits similar to dentin, and that in both the keratin hydrogel group and the calcium hydroxide group, root canals completely filled with osteodentin-like material were identified. Statistical analysis of the mineralization scores revealed no significant difference between the groups.

The lack of benefit may be related to the similar impact of the materials used. Calcium hydroxide inhibits osteoclast activity and stimulates tissue repair, promoting the formation of hydroxyapatite through the release of phosphate and calcium ions, thus demonstrating its power to induce the formation of mineralized tissue [52]. Keratin hydrogel favors cell viability, proliferation, and cell interaction, making it a promising platform for tissue regeneration [53]. Keratin has potential for healing due to its bioactivity and biocompatibility [54]. Fibrinogen, in turn, stimulates reparative cell activity and has angiogenic and anti-inflammatory properties that are essential for bone regeneration [55, 56].

With regard to inflammation, the experimental groups showed a reduction of 0.44 (95% CI: 0.21-0.92) in inflammation compared to the controls, with no significant heterogeneity between the studies (P=0.290). Removing the data from Liu *et*

al. [30], and Zhu *et al.* [18], would dilute the difference in favor of the scaffolds (P>0.05), and there was no significant publication bias (P=0.117). These results indicate a potential benefit of experimental treatments in reducing inflammation.

The literature emphasizes that the ideal biomaterial should have immunomodulatory properties, controlling the inflammatory response and stimulating tissue repair through the production of anti-inflammatory mediators [57]. Glucocorticoids such as dexamethasone have a strong anti-inflammatory action [58, 59]. Heparin coating and the controlled release of anti-inflammatory cytokines such as IL-6 and IL-10 have been effective in reducing fibrotic capsules and promoting tissue regeneration [60]. The combination of dexamethasone with copper reduces pro-inflammatory markers, while with magnesium it increases IL-10 expression [61]. Other anti-inflammatory agents, such as peptides derived from thymosin β-4 (SDKP) and laminin (C16), have reduced pro-inflammatory cytokines and phagocytic activity [62].

These advances highlight the importance of modulating the immune response to optimize the integration of biomaterials into the body, ensuring greater efficacy and longevity of implantations. In addition, the findings of this study corroborate previously observed results. With regard to the studies by Liu *et al.* [30] and Zhu *et al.* [18], both show that the agents used, respectively, demineralized bone matrix (DBM) and chitosan hydrogel with silver-doped bioactive glass, have anti-inflammatory properties. However, when compared to the others, these scaffolds showed a more delayed inflammatory response and were unable to completely control the inflammation.

Limitations of the study

One of the limitations of this study is that, of the 17 articles selected, only 13 could be included in the meta-analysis. The four studies that were only included in the systematic review had limitations related to the lack of quantitative analysis (Farzad-Mohajeri *et al.* [27]; Goldberg *et al.* [29]; Yun *et al.* [26]) or the use of a different measurement system from that commonly adopted by other studies (Han *et al.* [34]), which reported data as range (minimum–maximum) rather than mean and standard deviation, making comparison with other studies impossible. This emphasizes the importance of future research adopting standardized measurement systems to maximize inclusion in meta-analyses and strengthen the power of statistical associations.

Additionally, the exclusive use of animal models (rats and dogs) limits the applicability of the findings to humans, as key anatomical, immunological, and healing differences may influence outcomes [63]. Egger's and Begg's tests indicated significant publication bias for dentin thickness (P=0.001) and dentin bridge formation (P=0.044), suggesting an overestimation of effects. While the control groups included clinically established materials such as MTA and $Ca(OH)_2$, more recent alternatives like Biodentine, a bioactive dentin substitute with recognized clinical performance in vital pulp therapy [64], were not represented. High risk of bias in allocation concealment and blinding was identified in more than 45% of the included studies, and most studies had follow-up periods of 90 days or less, leaving long-term pulp vitality and complications (*e.g.*, necrosis, microleakage) unaddressed.

Conclusion

This systematic review/meta-analysis demonstrates that three-dimensional scaffolds have potential as direct pulp capping agents by enhancing cellular activity, dentin formation, and inflammation control. However, their effect on dentin bridge formation was limited and did not surpass that of clinically established materials such as MTA and $Ca(OH)_2$. Outcomes related to pulp tissue organization and the risk of dystrophic calcification also remain inconclusive. The low certainty of evidence and methodological variability among studies highlight the need for standardized experimental protocols and long-term evaluations. Despite these limitations, scaffold-based approaches offer a promising avenue to promote pulp regeneration and dentinogenesis, potentially contributing to the short-term preservation of pulp vitality and reducing the need for invasive treatments. Future research should focus on optimizing scaffold composition, refining the delivery of bioactive molecules, and conducting comparative studies with modern bioceramics to ensure consistent clinical outcomes. While these strategies show potential, further high-quality studies are essential before their routine clinical application in vital pulp therapy.

Data availability

This protocol for the systematic review was registered in the PROSPERO database (registration number CRD42024495369) and adheres to the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines.

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Conflict of interest

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Authors' contributions

Conceptualization: DAC; Methodology: DAC/ELCF/PGBS/GBQ; Formal Analysis and Investigation: GBQ/DAC/ELCF/PGBS/GAJC; Writing-Original draft preparation: GBQ/GAJC/DAC/JPMP/AVVN/NSRG/VPAS; Writing-review and editing: GBQ/DAC; Supervision: DAC/PGBS. All authors read and approved the final manuscript.

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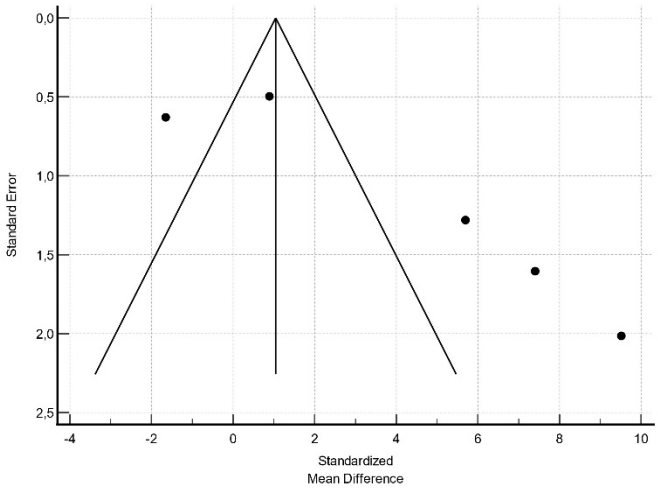
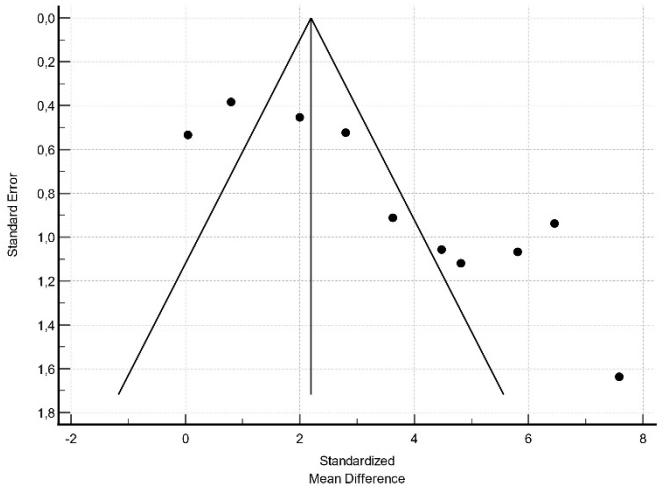
Supplementary File 1. Search Terms specific for each database and truncations

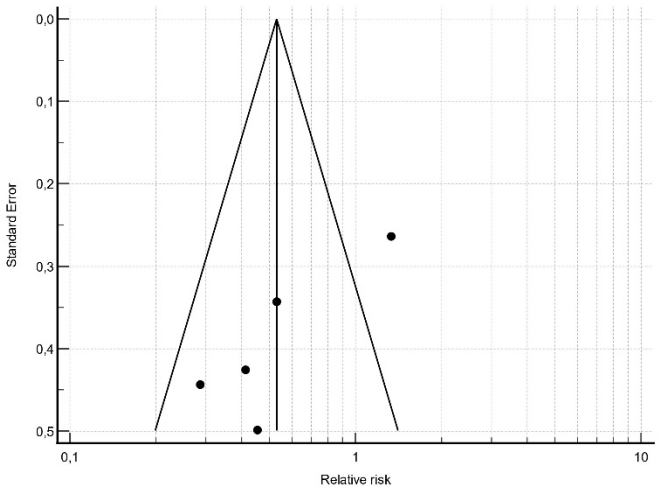
Eletronic Database	Search strategy used	Itens found (included/ found)
Keywords	Demineralized Dentin Matrix; Dental Pulp Capping; Scaffold; Hydrogels. "Dental Pulp Capping"[Mesh] = “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental” "Hydrogels"[Mesh] = “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”	
PubMed	(((((((("Dental Pulp Capping"[Mesh]) OR “Capping, Dental Pulp”) OR “Capping, Pulp”) OR “Pulp Capping”) OR “Pulp Capping, Dental”) OR “Cappings, Pulp”) OR “Pulp Cappings”) OR “Cappings, Dental Pulp”) OR “Dental Pulp Cappings”) OR “Pulp Cappings, Dental”) AND (“Demineralized Dentin Matrix” OR “Scaffold” OR (((((("Hydrogels"[Mesh]) OR “Hydrogel”) OR “In Situ Hydrogels”) OR “In Situ Hydrogel”) OR “Hydrogel, In Situ”) OR “Patterned Hydrogels”) OR “Patterned Hydrogel”) OR “Hydrogel, Patterned”)	69
Scopus	TITLE-ABS-KEY ("Dental Pulp Capping" OR “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental”) AND TITLE-ABS-KEY (“Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels" OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”)	83
Embase	("Dental Pulp Capping" OR “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental”) AND (“Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels" OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”)	53
Web of Science	TÓPICO: ((((((((("Dental Pulp Capping") OR “Capping, Dental Pulp”) OR “Capping, Pulp”) OR “Pulp Capping”) OR “Pulp Capping, Dental”) OR “Cappings, Pulp”) OR “Pulp Cappings”) OR “Cappings, Dental Pulp”) OR “Dental Pulp Cappings”) OR “Pulp Cappings, Dental”) AND (“Demineralized Dentin Matrix” OR “Scaffold” OR (((((((("Hydrogels") OR “Hydrogel”) OR “In Situ Hydrogels”) OR “In Situ Hydrogel”) OR “Hydrogel, In Situ”) OR “Patterned Hydrogels”) OR “Patterned Hydrogel”) OR “Hydrogel, Patterned”))	60
COCHRANE	ID Search Hits #1 "Dental Pulp Capping" OR “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental”:ti,ab,kw (Word variations have been searched #2 “Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels" OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”:ti,ab,kw (Word variations have been searched) #3 #1 AND #2	4
LIVIVO	("Dental Pulp Capping" OR “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental”) AND (“Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels" OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”)	195
LILACS	(tw:("Dental Pulp Capping" OR “Capping, Dental Pulp” OR “Capping, Pulp” OR “Pulp Capping” OR “Pulp Capping, Dental” OR “Cappings, Pulp” OR “Pulp Cappings” OR “Cappings, Dental Pulp” OR “Dental Pulp Cappings” OR “Pulp Cappings, Dental”)) AND (tw:(“Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels" OR “Hydrogel” OR “In Situ Hydrogels” OR “In Situ Hydrogel” OR “Hydrogel, In Situ” OR “Patterned Hydrogels” OR “Patterned Hydrogel” OR “Hydrogel, Patterned”))	0
Grey literature		
Google Scholar	Search #1. allintitle: "Dental Pulp Capping" Search #2. allintitle: “Demineralized Dentin Matrix” OR “Scaffold” OR "Hydrogels"	110



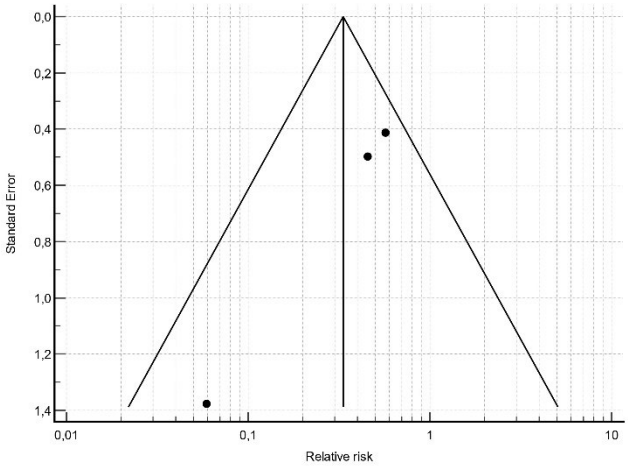
Supplementary file 2: Funnel plot of the cellularity



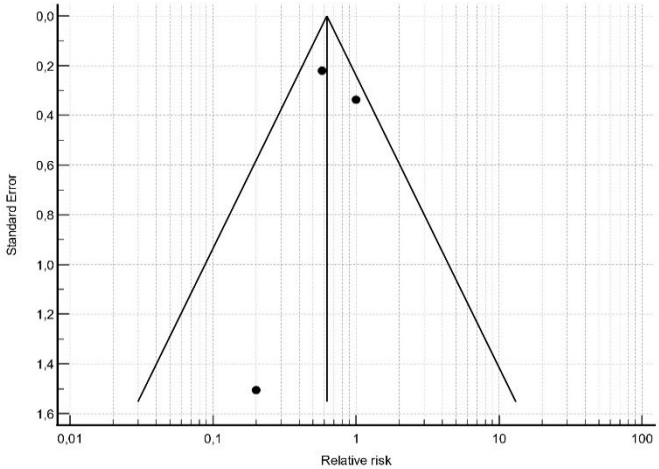
Supplementary file 3: Funnel plot of the dentin thickness



Supplementary file4: Funnel plot of the dentin bridging



Supplementary file 5: Funnel plot of the dystrophic calcification



Supplementary material 6. Funnel plot of the inflammation