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REVIEW

Regenerative Potential of Exosomes from 3D Spheroid-Cultured Stem Cells Compared with 2D Culture in Dentin–Pulp Regeneration: A Systematic Review

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Purpose: This systematic review aimed to evaluate the current preclinical evidence on exosomes derived from three-dimensional stem cell culture systems and their direct or indirect relevance to dentin–pulp regeneration. Exosomes derived from stem cells have emerged as promising mediators of tissue regeneration because of their ability to transfer bioactive molecules that regulate cellular communication and tissue repair. Recent studies suggest that exosomes obtained from three-dimensional (3D) spheroid cultures may exhibit enhanced regenerative properties compared with those derived from conventional two-dimensional cultures.

Methods: A systematic review was conducted following the PRISMA 2020 guidelines. Electronic searches were performed in PubMed, Scopus, and ProQuest databases to identify relevant studies investigating exosomes derived from 3D cell culture systems with potential relevance to dentin–pulp regeneration. Studies were screened according to predefined inclusion and exclusion criteria. Data extraction focused on cell sources, exosome isolation methods, characterization techniques, and biological effects related to dental pulp regeneration.

Results: Ten studies met the inclusion criteria. The included studies demonstrated that exosomes derived from 3D spheroid cultures may enhance angiogenesis, support odontogenic differentiation, reduce inflammatory responses, and promote regenerative tissue repair. Several studies also reported increased expression of regenerative markers and improved cellular proliferation when compared with exosomes derived from conventional two-dimensional cultures. However, only a limited number of studies directly investigated dentin–pulp-related outcomes, while several provided indirect mechanistic evidence from non-dental preclinical models.

Conclusion: Exosomes derived from three-dimensional culture systems show promising potential as a cell-free therapeutic strategy for dentin–pulp regeneration. These vesicles may improve tissue repair through enhanced biological activity. However, the current evidence remains mainly preclinical, heterogeneous, and partly indirect. Further standardized experimental studies and clinical investigations are required to validate their translational application in regenerative endodontic therapy.

Plain Language Summary: Damage to dental pulp is commonly treated with root canal therapy, which removes infected tissue but does not restore the natural living function of the tooth. Regenerative dentistry aims to help repair the soft tissue and hard tissue inside the tooth. Exosomes are tiny particles released by cells. They carry helpful signals that can guide nearby cells to repair tissue, reduce inflammation, and support healing. Stem cell-derived exosomes have been studied as a possible treatment for dental pulp repair. Recent studies suggest that cells grown in rounded three-dimensional groups may release exosomes with stronger repair activity than cells grown in flat two-dimensional layers. This systematic review examined current studies on exosomes from three-dimensional stem cell cultures and their possible role in dentin–pulp regeneration. The findings suggest that these exosomes may help form new blood

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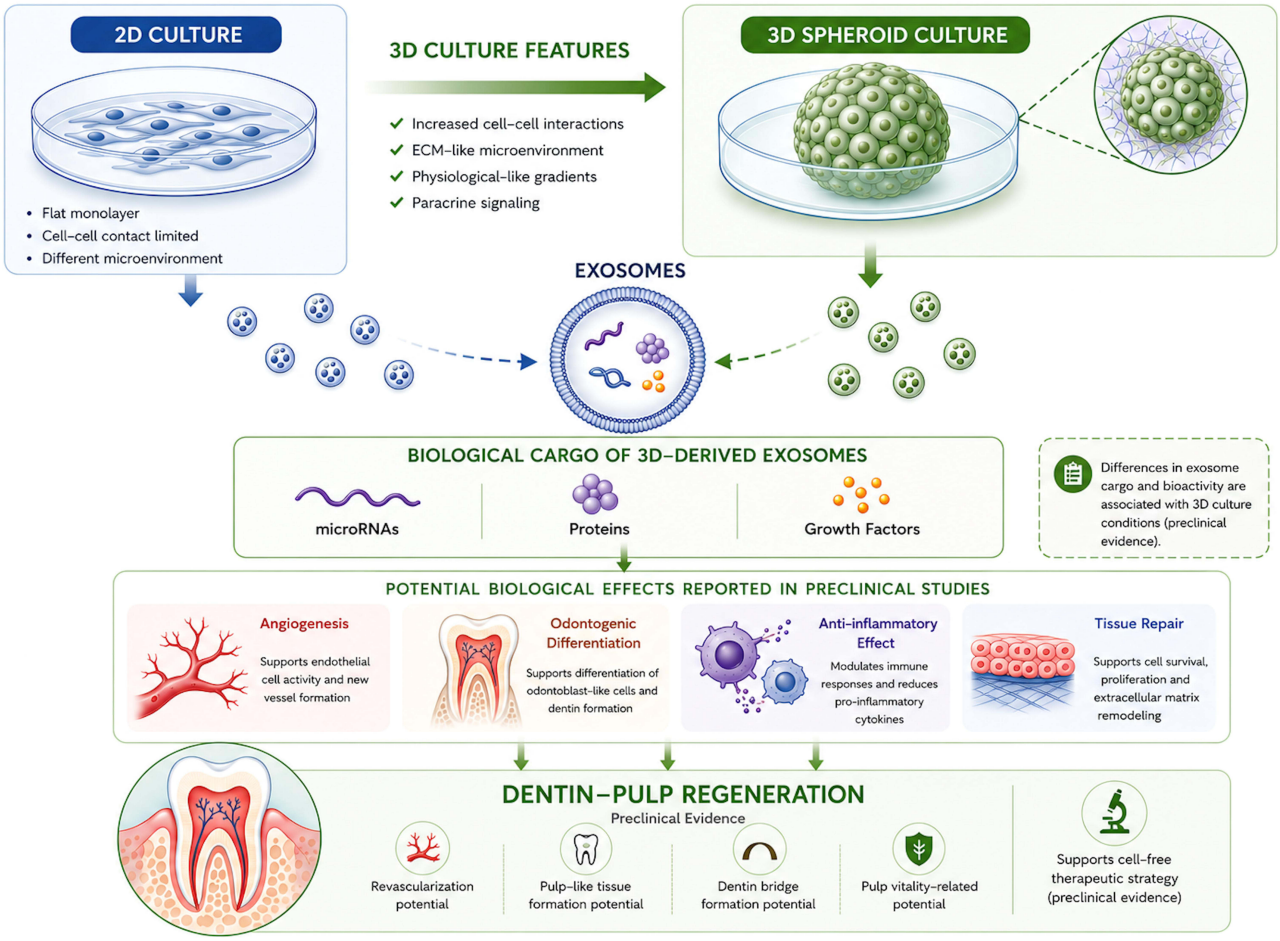
vessels, reduce inflammation, and support tissue repair. Some studies also showed effects related to dentin-forming cells. However, most available studies were performed in laboratories or animal models, and some were only indirectly related to dental pulp regeneration. More standardized studies and clinical research are needed before this approach can be used routinely in dental treatment.

Keywords: exosomes, three-dimensional culture, dental pulp stem cells, dentin–pulp regeneration, regenerative endodontics, extracellular vesicles

Graphical Abstract



Introduction

Regenerative endodontics has emerged as a promising therapeutic strategy aimed at restoring the biological function of the dentin–pulp complex rather than merely replacing diseased or damaged tissue with synthetic filling materials that do not fully restore the physiological vitality of the pulp tissue. Conventional endodontic treatment, including root canal therapy, is effective in eliminating infection and relieving symptoms, but it does not re-establish the physiological vitality, sensory function, or reparative capacity of the dental pulp.[1] Regenerative endodontics is based on the triad of tissue engineering, which includes stem cells, scaffolds, and bioactive signaling molecules that collectively promote pulp tissue regeneration and revascularization.[2]

Stem cell–based approaches have been extensively investigated in regenerative dentistry because of their potential to promote angiogenesis, modulate inflammation, and induce differentiation into odontoblast-like cells.[2] However, direct stem cell transplantation remains associated with several challenges, including limited cell survival, possible immune reactions, ethical concerns, and regulatory barriers that may hinder translation into routine clinical practice.[3,4] These limitations have stimulated growing interest in cell-free therapeutic strategies.

Among these, exosomes have gained considerable attention as biologically active extracellular vesicles capable of mediating intercellular communication. Exosomes are nanosized membrane-bound vesicles that carry proteins, lipids, messenger RNAs, and microRNAs, thereby regulating multiple processes involved in tissue repair and regeneration.[5,6] According to the Minimal Information for Studies of Extracellular Vesicles (MISEV2018) guidelines, exosomes are generally characterized based on their size, morphology, and the expression of markers such as CD63, CD81, and CD9.[7] Extracellular vesicles (EVs) are broadly classified into exosomes, microvesicles, and apoptotic bodies based on their size and biogenesis. Compared with other EVs, exosomes exhibit a more homogeneous size distribution and more stable bioactive cargo, making them particularly attractive for regenerative applications.[3] In the context of regenerative dentistry, exosomes derived from mesenchymal stem cells, particularly dental pulp stem cells, have demonstrated the ability to enhance angiogenesis, reduce inflammation, and promote odontogenic differentiation.[8,9]

Most studies investigating regenerative exosomes have traditionally used two-dimensional (2D) culture systems. However, 2D culture conditions fail to recapitulate the complex three-dimensional (3D) microenvironment found in living tissues, which may influence cell behavior, signaling pathways, and secreted vesicle composition.[10] In contrast, 3D culture systems such as spheroids, scaffold-based cultures, and hollow-fiber bioreactors may better reproduce certain aspects of the in vivo cellular niche by promoting cell–cell and cell–matrix interactions, extracellular matrix deposition, and physiologically relevant gradients.[11–13] Emerging evidence suggests that exosomes derived from 3D culture systems may possess distinct molecular cargo and potentially enhanced regenerative bioactivity compared with exosomes derived from conventional 2D cultures.[14–17]

Despite these promising findings, the role of 3D culture–derived exosomes in dentin–pulp regeneration has not yet been systematically synthesized. Therefore, this systematic review aimed to evaluate the current evidence regarding exosomes derived from three-dimensional culture systems in the context of dentin–pulp regeneration. This review specifically examined whether exosomes derived from 3D stem cell culture systems demonstrate regenerative effects relevant to dentin–pulp regeneration and how these effects compare with those of conventional 2D culture-derived exosomes. Both direct dentin–pulp-related studies and indirect mechanistic preclinical studies relevant to regenerative processes were considered in this review to provide a broader understanding of the biological effects of 3D culture-derived exosomes. Furthermore, this review aimed to highlight translational implications, current limitations, and future research directions for exosome-based approaches in regenerative endodontics.

Materials and Methods

Study Design

This study was conducted as a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[18] Because of the anticipated heterogeneity among included studies, a narrative synthesis approach was planned. The review protocol was prospectively registered in the Open Science Framework (OSF; DOI: 10.17605/OSF.IO/4EKXC). The review design followed a structured process consisting of literature search, study selection, data extraction, quality appraisal, and narrative synthesis.

Search Strategy

A comprehensive electronic search strategy was developed to identify studies investigating exosomes derived from three-dimensional culture systems and their potential direct or indirect relevance to dentin–pulp regeneration. The databases PubMed, Scopus, and ProQuest were systematically searched for articles published between January 2015 and April 2025. No language restrictions were applied during the initial search, although only studies published in English were included in the final review.

Table 1 Databases and Search Strategies Used in the Systematic Review

Database	Search Strategy
ProQuest	NOFT(exosome OR "extracellular vesicle" OR EV) AND NOFT("3D culture" OR "three-dimensional" OR spheroid OR organoid OR "aggregate culture") AND NOFT("dental pulp" OR dentin OR "dentin-pulp complex" OR odontogenesis OR "endodontic regeneration" OR "tooth regeneration")
PubMed	(exosome*[tiab] OR "Extracellular Vesicles"[tiab]) AND (spheroid*[tiab] OR "3D culture"[tiab] OR "three dimensional"[tiab] OR organoid*[tiab] OR "aggregate culture"[tiab]) AND ("dental pulp"[tiab] OR dentin[tiab] OR "dentin-pulp complex"[tiab] OR odontogen*[tiab] OR "endodontic regeneration"[tiab] OR "tooth regeneration"[tiab])
Scopus	TITLE-ABS-KEY ("exosome" OR "extracellular vesicle" OR EV) AND TITLE-ABS-KEY ("3D culture" OR "three-dimensional" OR "spheroid" OR "organoid" OR "aggregate culture") AND TITLE-ABS-KEY ("dental pulp" OR dentin OR "dentin-pulp complex" OR odontogen OR "endodontic regeneration" OR "tooth regeneration")

The search strategy combined controlled vocabulary and free-text terms related to exosomes, extracellular vesicles, and three-dimensional culture systems. Search terms included "exosome", "extracellular vesicle", "EV", "3D culture", "three-dimensional", "spheroid", "scaffold", "organoid", and "aggregate culture", combined with dentin–pulp-related terms including "dental pulp", "dentin", "dentin-pulp complex", "odontogenesis", "endodontic regeneration", and "tooth regeneration". Boolean operators (AND, OR) were used to optimize sensitivity and specificity. The detailed search strategy for each database is presented in Table 1.

Eligibility Criteria

Studies were selected according to predefined inclusion and exclusion criteria based on the PICOS framework (Population, Intervention, Comparator, Outcomes, and Study design). The comparator of interest included conventional two-dimensional (2D) culture-derived exosomes when available. Studies were included if they:

1. Investigated exosomes or extracellular vesicles derived from 3D culture systems
2. Involved cell sources relevant to regenerative applications, including mesenchymal stem cells or dental-derived stem cells
3. Evaluated biological outcomes relevant to dentin–pulp regeneration, such as angiogenesis, odontogenic differentiation, anti-inflammatory effects, pulp vitality, or tissue repair. Studies reporting indirect mechanistic evidence relevant to regenerative processes, including angiogenesis, anti-inflammatory activity, or mineralization-related pathways, were also considered when direct dentin–pulp outcomes were limited.
4. Used in vitro, in vivo, or preclinical experimental designs
5. Were published in English

Studies were excluded if they:

1. Did not involve exosome isolation or characterization
2. Did not use a 3D culture system
3. Did not report regenerative or biologically relevant outcomes
4. Were conference abstracts, editorials, reviews, or duplicate publications

Study Selection

The search results were exported into Rayyan for screening and duplicate removal.[19] After duplicate removal, titles and abstracts were independently screened for relevance by reviewers using the predefined eligibility criteria. Potentially eligible studies underwent full-text assessment. Any disagreements during the screening process were resolved through discussion until consensus was achieved.

Search Outcome

The initial search across PubMed, Scopus, and ProQuest identified 882 records. After removal of 62 duplicates, 820 unique records remained for title and abstract screening. Of these, 65 records were selected for full-text assessment. Following full-text review, 55 articles were excluded because they did not meet the inclusion criteria, primarily due to the absence of 3D culture methodology, lack of exosome isolation, or outcomes unrelated to dentin–pulp regeneration. Ultimately, 10 studies were included in the final qualitative synthesis. The study selection process is shown in Figure 1.

Data Extraction

Data extraction was conducted independently by two reviewers using a standardized form. The following variables were extracted from each included study: author and year of publication, cell source, study design, 3D culture method, exosome isolation method, characterization techniques, biological outcomes, and conclusions relevant to dentin–pulp regeneration. When discrepancies occurred, the full texts were re-examined and resolved by discussion until agreement was reached.

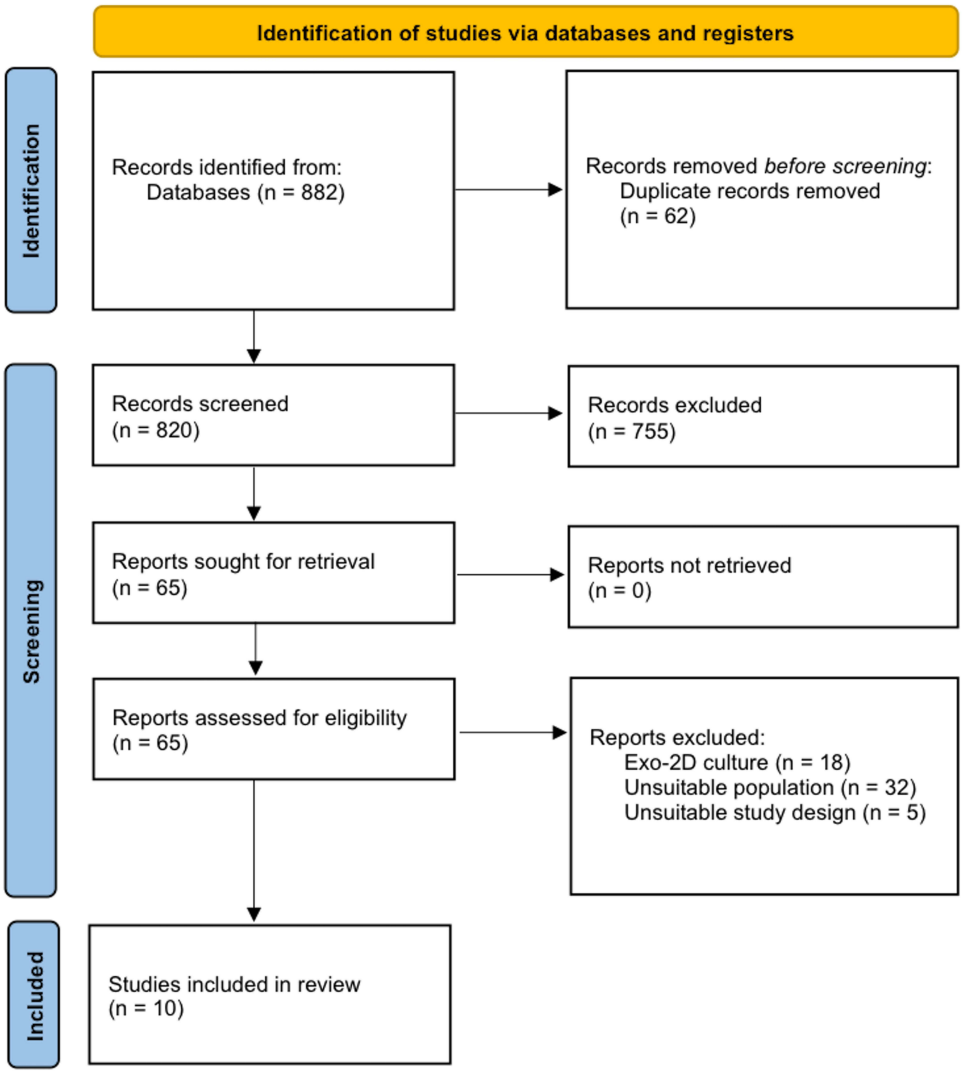


Figure 1 PRISMA 2020 flow diagram illustrating identification, screening, eligibility assessment, and inclusion of studies in this systematic review.

Quality Assessment

The methodological quality of the included studies was assessed to determine the reliability of the available evidence. For in vivo studies, SYRCLE's risk of bias tool was applied.[20] This tool evaluates domains such as sequence generation, baseline characteristics, allocation concealment, random housing, blinding, incomplete outcome data, selective outcome reporting, and other sources of bias.

For in vitro studies, a modified SYRCLE-based framework was used, focusing on domains relevant to laboratory-based experimental designs, including clarity of study objectives, randomization of experimental groups, blinding, outcome assessment, and selective reporting.[21] Each domain was rated as "Yes" for low risk of bias, "No" for high risk of bias, or "Unclear" where reporting was insufficient.

Data Synthesis

Because of the heterogeneity across studies in terms of cell sources, 3D culture platforms, exosome isolation methods, and reported outcomes, a meta-analysis was not considered appropriate. Therefore, a narrative synthesis was performed following the principles of the Synthesis Without Meta-analysis (SWiM) reporting guideline where applicable. The synthesis primarily applied structured thematic narrative interpretation and direction-of-effect assessment to summarize heterogeneous regenerative outcomes across the included studies.[22] The findings were organized into structured tables summarizing study characteristics and biological outcomes. Results were synthesized thematically according to key regenerative processes including angiogenesis, odontogenesis, pulp vitality, and anti-inflammatory activity. Risk of bias findings were integrated into the interpretation of the evidence.

Results

Study Characteristics

A total of 10 studies met the eligibility criteria and were included in this review. The included studies were published between 2018 and 2025 and comprised in vitro, in vivo, and combined preclinical designs. Cell sources investigated included dental pulp stem cells, bone marrow mesenchymal stem cells, umbilical cord mesenchymal stem cells, human embryonic stem cells, and other mesenchymal stem cell populations.

Various 3D culture approaches were used across the included studies, including spheroid formation, scaffold-based culture systems, hypoxia-preconditioned spheroids, 3D aggregates, and hollow-fiber bioreactors. Exosomes were isolated primarily using ultracentrifugation or tangential flow filtration and were commonly characterized using transmission electron microscopy, nanoparticle tracking analysis, and Western blotting. A detailed summary of the methodological characteristics of the included studies is presented in Table 2.

This table summarizes the methodological aspects of the ten included studies, including cell sources, study type, 3D culture platforms, isolation methods, and characterization techniques used for exosome analysis.

The proposed relationship between 3D spheroid formation, exosome secretion, and the biological effects relevant to dentin–pulp regeneration is illustrated in Figure 2.

Table 2 Characteristics of Included Studies Investigating 3D-Culture-Derived Exosomes for Dentin–Pulp Regeneration

Author/Year	Cell Source	Study Type	3D Culture Method	Exosome Isolation	Characterization
Faruqu et al (2021)	UC-MSC	In vitro	Serum-free spheroid	Ultracentrifugation	TEM, NTA, WB
Gao et al (2021)	BMSC	In vitro + in vivo	3D spheroid	Ultracentrifugation	TEM, NTA, WB
Haraszti et al (2018)	MSC	In vitro	3D bioreactor + TFF	Tangential Flow Filtration	TEM, NTA, WB
Chen et al (2025)	BMSC	In vitro + in vivo	3D cultivation + enrichment (HGF)	TFF	TEM, NTA, WB
Toghiani et al (2024)	WJ-MSC	In vitro + in vivo	Hypoxia-preconditioned spheroid	Ultracentrifugation	TEM, NTA, WB
Yan et al (2023)	UC-MSC	In vitro + in vivo	3D porous scaffold	TFF	TEM, NTA, WB
Guo et al (2022)	SHED aggregates / DPSCs	In vitro + in vivo	3D aggregates	Ultracentrifugation	TEM, NTA, WB, qPCR
Yan and Wu (2020)	UC-MSC	In vitro + in vivo	Hollow-fiber bioreactor	Bioreactor + TFF	TEM, NTA, WB
Wang et al (2021)	hESC	In vitro + in vivo	3D spheroid	Ultracentrifugation	TEM, NTA, WB, miRNA
Xu et al (2022)	hUC-MSC	In vitro + in vivo	3D spheroid	Ultracentrifugation	TEM, NTA, WB, miRNA

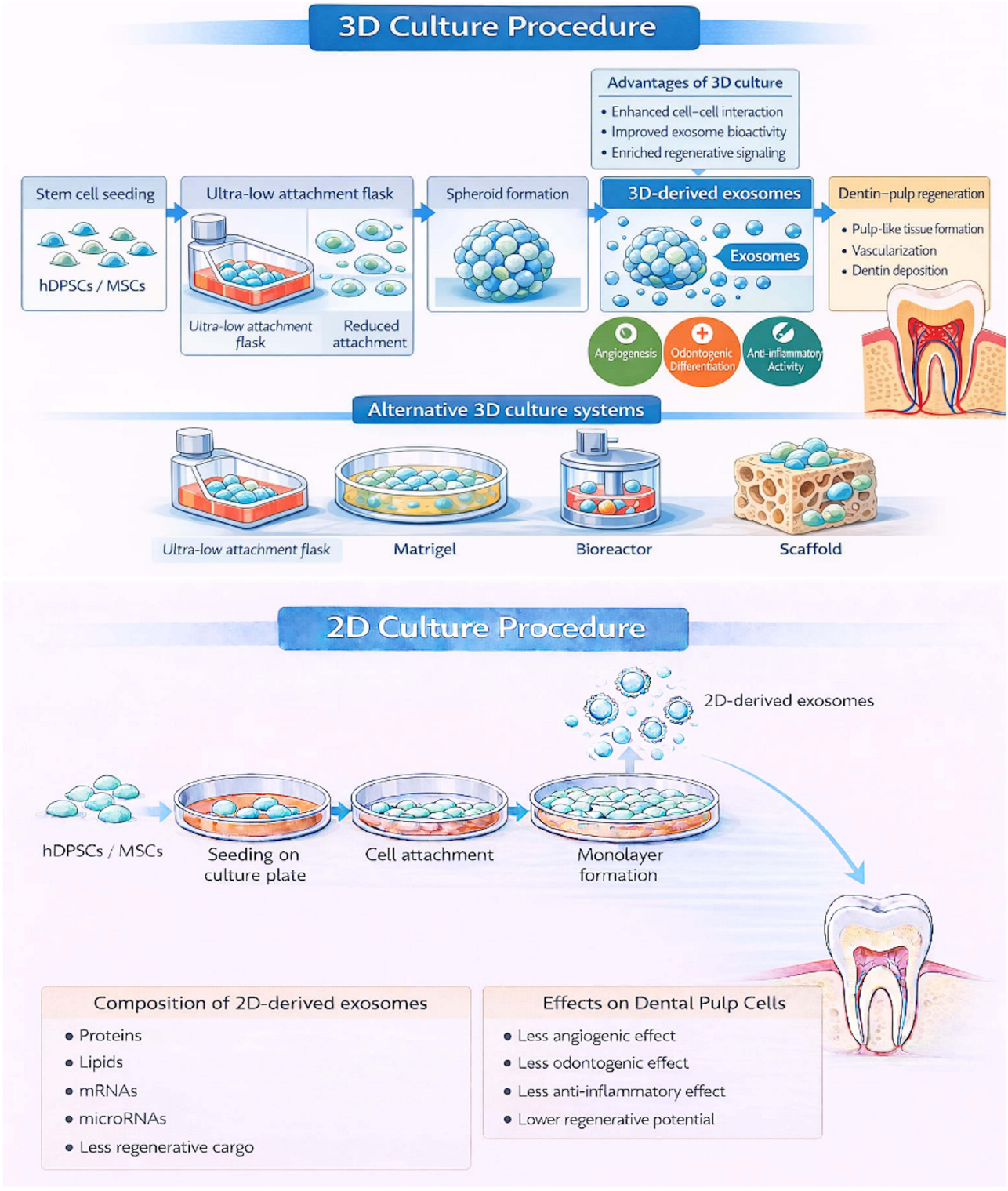


Figure 2 Schematic drawing of exosome production in three-dimensional (3D) and two-dimensional (2D) stem cell culture systems. The upper panel shows 3D culture using ultra-low attachment flasks and alternative techniques, including Matrigel, scaffold, and bioreactor systems, leading to spheroid formation and potentially enhanced exosome bioactivity. The lower panel illustrates conventional 2D monolayer culture, resulting in exosomes with comparatively lower reported regenerative activity. Differences in composition and biological effects on dentin–pulp regeneration are highlighted. The schematic drawing was generated with the assistance of ChatGPT (OpenAI).

Biological Outcomes and Relevance to Dentin–Pulp Regeneration

The included studies generally suggested that exosomes derived from 3D culture systems may exhibit enhanced regenerative potential compared with exosomes derived from conventional 2D cultures. The reported biological outcomes included angiogenesis, odontogenic differentiation, anti-inflammatory effects, and tissue repair.

Several studies demonstrated that 3D-derived exosomes promoted angiogenesis by enhancing endothelial proliferation, migration, and vascularization, in some cases through HMGB1/AKT signaling pathways.[10,11,23] Other studies reported that exosomes from 3D spheroids and related systems enhanced odontogenic differentiation and dentin-associated regeneration, either directly in dental pulp-related cells or indirectly through mechanistic pathways relevant to mineralized tissue formation.[4,10,11,24]

Anti-inflammatory activity was also reported in studies using hypoxia-conditioned spheroids and enriched exosome preparations, suggesting that 3D-derived exosomes may support regenerative healing by modulating inflammatory responses.[22,25–27] In addition, several studies supported the potential translational relevance of 3D culture systems by demonstrating improved exosome yield, bioactivity, and scalability, particularly in bioreactor-based production platforms.[6,28] The main biological findings and their relevance to dentin–pulp regeneration are summarized in Table 3.

The main biological findings and their relevance to dentin–pulp regeneration are summarized in Table 3. Overall, the included studies demonstrated enhanced angiogenic, osteogenic, anti-inflammatory, anti-fibrotic, and regenerative-supportive effects of exosomes derived from 3D culture systems, although several studies provided indirect mechanistic or translational evidence rather than direct dentin–pulp outcomes.

Risk of Bias

The overall methodological quality of the included studies was considered acceptable, although several domains showed moderate risk of bias. For in vitro studies, most investigations clearly reported their objectives, exosome isolation and characterization methods, and measured outcomes. However, randomization, allocation concealment, and blinding were frequently rated as unclear or high risk due to insufficient reporting.

For in vivo studies, methodological rigor was also moderate overall. While several studies adequately described baseline comparability and outcome assessment, many lacked clear descriptions of randomization, allocation concealment, and blinding procedures. Selective reporting was generally judged to be at low risk across most studies. The detailed risk of bias assessments are presented in Tables 4 and 5, and the visual summary is shown in Figure 3.

For the in vivo studies (Table 5), the risk of bias was generally moderate. While several studies reported baseline comparability and outcome assessment adequately, many domains such as allocation concealment, randomization, and

Table 3 Biological Outcomes and Relevance of Included Studies

Author/Year	Biological Outcomes	Key Findings	Relevance to Pulp–Dentin Regeneration
Faruqu et al (2021)	Fibroblast proliferation, migration	Exosomes enhanced fibroblast activity	Supportive via stromal activation
Gao et al (2021)	Angiogenesis (HMGB1/AKT pathway)	Increased endothelial proliferation and migration	Indirect relevance via angiogenesis
Haraszti et al (2018)	Yield and potency	3D-TFF exosomes showed higher yield and bioactivity	Methodological support for scalable production
Chen et al (2025)	Barrier protection, anti-inflammation	HGF-enriched exosomes protected lung epithelium	Indirect mechanistic relevance
Toghiani et al (2024)	Renal restoration, angiogenesis	Hypoxia-3D exosomes promoted renal cell survival	Supportive mechanistic evidence
Yan et al (2023)	Osteochondral repair	Enhanced osteochondral regeneration in vivo	Indirect pulp-dentin relevance
Guo et al (2022)	Osteogenesis in DPSCs	Exosome-shuttled TFAM mRNA enhanced osteogenic differentiation	Directly relevant to pulp–dentin regeneration
Yan and Wu (2020)	Osteochondral regeneration	Hollow-fiber 3D exosomes improved repair	Indirect regenerative relevance
Wang et al (2021)	Anti-fibrosis (TGFβRII-SMADs)	hESC exosomes attenuated liver fibrosis	Indirect mechanistic evidence
Xu et al (2022)	Anti-fibroblast activation	let-7i-5p exosomes suppressed TGFBR1 signaling	Supportive mechanistic pathway

Table 4 Risk of Bias for in vitro Studies (Modified SYRCLE's RoB Tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Faruqu et al (2021)	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Gao et al (2021)	No	Yes	Yes	No	Yes	Yes	No	No	Unclear	Yes
Haraszti et al (2018)	No	Yes	Yes	Yes	Yes	Yes	No	Unclear	Unclear	Yes
Chen et al (2025)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Toghiani et al (2024)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Unclear	Yes
Yan et al (2023)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Guo et al (2022)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Yan and Wu (2020)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Wang et al (2021)	No	Yes	Yes	Yes	Yes	Yes	No	Unclear	Unclear	Yes
Xu et al (2022)	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

Notes: Items were adapted from SYRCLE's risk of bias tool for laboratory-based studies. Item 1, randomization of experimental groups; Item 2, baseline comparability; Item 3, clarity of intervention or exposure; Item 4, standardized culture conditions; Item 5, clarity of exosome isolation and characterization; Item 6, clarity of outcome measurement; Item 7, blinding of outcome assessment; Item 8, incomplete outcome data; Item 9, selective outcome reporting; Item 10, other sources of bias.

Table 5 Risk of Bias for in vivo Studies (SYRCLE's RoB Tool)

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Gao et al (2021)	Yes	Yes	No	Yes	Unclear	Yes	No	Unclear	Unclear	Yes
Chen et al (2025)	Yes	No	No	Yes	Unclear	Yes	No	Yes	Yes	Yes
Toghiani et al (2024)	No	Unclear	No	Unclear	Unclear	Yes	No	Yes	Yes	Yes
Yan et al (2023)	Yes	Yes	Yes	Yes	No	Unclear	No	Yes	Yes	Yes
Guo et al (2022)	Yes	Yes	Unclear	Yes	No	Yes	No	Unclear	Unclear	Yes
Yan and Wu (2020)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Wang et al (2021)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear
Xu et al (2022)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Unclear

Notes: Items were based on SYRCLE's risk of bias tool. Item 1, sequence generation; Item 2, baseline characteristics; Item 3, allocation concealment; Item 4, random housing; Item 5, blinding of caregivers and/or investigators; Item 6, random outcome assessment; Item 7, blinding of outcome assessment; Item 8, incomplete outcome data; Item 9, selective outcome reporting; Item 10, other sources of bias.

blinding were marked as *Unclear* or *No*. This highlights the challenges of methodological rigor in preclinical animal studies. Nevertheless, selective outcome reporting was judged as low risk across nearly all studies, supporting the reliability of the reported findings.

Discussion

This systematic review highlights the emerging potential of exosomes derived from three-dimensional culture systems as a cell-free regenerative strategy for dentin–pulp regeneration. Across the included studies, 3D culture–derived exosomes generally demonstrated potentially enhanced biological effects compared with exosomes derived from conventional 2D cultures, particularly in terms of angiogenesis, odontogenic differentiation, anti-inflammatory activity, and overall regenerative bioactivity.[4,6,8,10,11,23–28] These findings are consistent with the concept that 3D cellular microenvironments more closely resemble physiological tissue conditions and consequently influence the composition and therapeutic potency of secreted exosomes.[5,15–17]

One of the major strengths identified across the reviewed studies was the diversity of stem cell sources used for exosome production. These included dental pulp stem cells, stem cells from exfoliated deciduous teeth, bone marrow mesenchymal stem cells, and umbilical cord-derived mesenchymal stem cells. Although all of these cell populations generated bioactive exosomes, those derived from dental or dental-related stem cells appeared to have the most direct translational relevance to regenerative endodontics.[4,10,11] This is particularly important because tissue-specific stem cell sources may produce exosomes with cargo more closely aligned to the biological requirements of dentin–pulp repair.

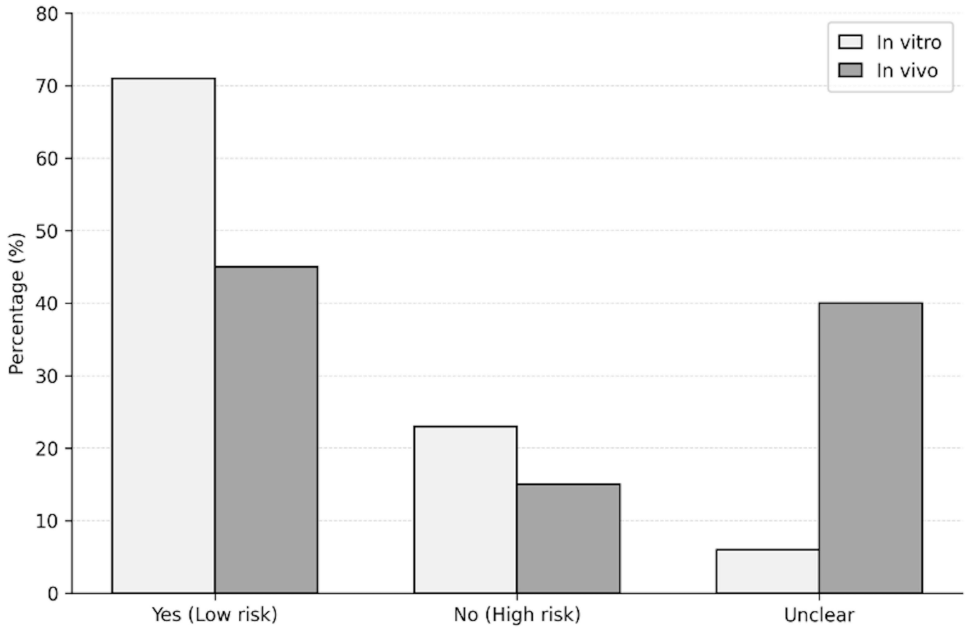


Figure 3 Risk of bias summary for included studies.

The potentially enhanced effects of 3D-derived exosomes may be attributable to the microenvironmental conditions generated in spheroids, scaffolds, and bioreactor systems. These conditions enhance cell–cell communication, extracellular matrix interactions, and the establishment of metabolic gradients that influence exosome secretion and cargo composition.[5,6,15,16] Several studies suggested that 3D cultures enrich exosomes with regenerative molecules, including pro-angiogenic and pro-odontogenic microRNAs and proteins, thereby contributing to stronger therapeutic effects.[8,10,11]

Angiogenesis emerged as one of the most consistently reported regenerative outcomes across the included studies. This finding is particularly relevant to dentin–pulp regeneration because successful regeneration of pulp tissue requires adequate revascularization to support nutrient delivery, oxygenation, and long-term tissue viability.[10,11] Exosomes derived from 3D cultures enhanced endothelial migration and proliferation, supporting the hypothesis that these vesicles may facilitate microvascular formation in regenerating pulp-like tissues.[10,11,23]

In addition to angiogenesis, several included studies indicated improved odontogenic or mineralization-related responses. These outcomes suggest that 3D culture–derived exosomes may contribute not only to soft tissue repair but also to dentin-associated regeneration by modulating odontogenic differentiation pathways.[4,10,11,24] This dual action is of substantial interest for regenerative endodontic therapy, where coordinated vascularization and odontoblast-like differentiation are both essential.

Despite these promising findings, several limitations must be considered. First, the number of included studies remains relatively small, reflecting the novelty of this field. Second, there was substantial methodological heterogeneity among studies in terms of cell sources, culture platforms, exosome isolation methods, characterization protocols, and experimental models. This heterogeneity limited direct comparison across studies and prevented quantitative meta-analysis. Third, most of the evidence originated from in vitro or preclinical animal studies, with no clinical trials available at the time of review. As a result, direct translation into clinical endodontic practice remains preliminary.

Risk of bias assessment further indicated that many studies did not clearly report randomization, allocation concealment, or blinding, which may increase the possibility of overestimating positive effects.[20,21] Future studies should therefore adopt more rigorous reporting standards and standardized methodologies to improve reproducibility and comparability. Standardization is especially needed in exosome isolation and characterization, consistent with broader recommendations in extracellular vesicle research. Most included studies reported core extracellular vesicle characterization methods consistent with MISEV2018 recommendations, including transmission electron microscopy, nanoparticle tracking analysis, and Western blotting, although reporting completeness varied across studies.[2,12,14,20]

Overall, the current evidence suggests that exosomes derived from 3D culture systems represent a promising acellular strategy for dentin–pulp regeneration. However, further well-designed preclinical studies and early-phase clinical investigations are required to confirm their safety, efficacy, and translational feasibility. Nevertheless, the certainty of the available evidence should be considered low to moderate because of methodological heterogeneity, indirect mechanistic evidence, moderate risk of bias, and the predominance of preclinical studies.

Conclusion

This systematic review demonstrates that exosomes derived from three-dimensional culture systems, including spheroid, scaffold-based, and bioreactor-associated platforms, show promising regenerative potential for dentin–pulp regeneration. Compared with exosomes derived from conventional 2D cultures, 3D culture–derived exosomes were associated with enhanced angiogenic and odontogenic-related responses, anti-inflammatory activity, and regenerative-supportive effects in preclinical studies. These effects support their potential as a cell-free therapeutic approach in regenerative endodontics. No included study directly evaluated clinical endodontic outcomes in human subjects.

Nevertheless, the available evidence remains limited by methodological heterogeneity, moderate risk of bias, and the predominance of in vitro and preclinical models. Future research should focus on standardizing exosome production and characterization protocols, improving methodological rigor, and advancing translational studies, including clinical trials, to validate the therapeutic potential of 3D culture–derived exosomes in dental practice. Therefore, the current evidence should be interpreted cautiously because most available studies remain preclinical and methodologically heterogeneous.

Abbreviations

3D, three-dimensional; BMSC, bone marrow mesenchymal stem cell; DPSC, dental pulp stem cell; EV, extracellular vesicle; hESC, human embryonic stem cell; NTA, nanoparticle tracking analysis; OSF, Open Science Framework; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RoB, risk of bias; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation; TEM, transmission electron microscopy; TFF, tangential flow filtration; UC-MSC, umbilical cord mesenchymal stem cell; WB, Western blot; MSC, mesenchymal stem cell.

Use of Artificial Intelligence

AI-assisted tools, including ChatGPT (OpenAI), were used only for limited editorial support, estimated at less than 10% of the manuscript preparation process, specifically for English grammar refinement and assistance in preparing the graphical abstract and schematic drawing. No AI tools were used for data generation, data analysis, interpretation of findings, or decision-making. All scientific content was developed, reviewed, and approved by the authors, who take full responsibility for the manuscript.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

Ethical approval was not required for this study because this work is a systematic review of previously published literature and did not involve direct research on human participants or animals.

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Author Contributions

All authors made substantial contributions to the conception and design of the study, acquisition of data, analysis and interpretation of data, and drafting or critical revision of the manuscript. All authors agreed on the journal to which the

article will be submitted, reviewed and approved all versions of the manuscript, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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