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Original Research Article

Enhancing Odontogenic Differentiation and In Vitro Biomineralization Using Hybrid Chitosan/Gelatin/Nanohydroxyapatite Scaffolds: A Systematic Review

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Abstract: ***Aim:*** To systematically evaluate the effect of hybrid chitosan/gelatin/nanohydroxyapatite (CS/Gel/nHAp) scaffolds on odontogenic differentiation of dental pulp stem cells and in vitro biomineralization. ***Materials and Methods:*** Electronic searches were conducted in PubMed, Scopus, ScienceDirect and Google Scholar up to April 2024 in accordance with PRISMA guidelines. In vitro human and animal studies assessing odontogenic differentiation and mineralization outcomes using CS/Gel/nHAp scaffolds were included. Risk of bias was assessed using the QUIN tool and SYRCLE's risk of bias tool. ***Results:*** Seven studies met the inclusion criteria. CS/Gel/nHAp scaffolds consistently demonstrated enhanced cell adhesion, proliferation, upregulation of odontogenic markers (DSPP, DMP1, ALP and RUNX2) and increased mineralized matrix deposition compared with control scaffolds or cells cultured without scaffolds. ***Conclusions:*** Within the limitations of the included studies, CS/Gel/nHAp hybrid scaffolds exhibit promising odontogenic and biomineralization potential, suggesting their suitability as biomimetic platforms for dentin–pulp regeneration. ***Clinical relevance:*** CS/Gel/nHAp scaffolds may represent a future regenerative strategy for dentin–pulp complex reconstruction in periodontal and restorative therapy.

Keywords: Chitosan; Gelatin; Nanohydroxyapatite; Dental pulp stem cells; Odontogenic differentiation.

INTRODUCTION

The regeneration of dental tissues represents a major challenge and opportunity in regenerative medicine, particularly in the management of craniofacial trauma, congenital anomalies, caries-induced pulp necrosis, and periodontal disease. Conventional dental treatments are largely reparative, focusing on symptom relief or functional restoration rather than true biological regeneration. As a result, the native structural, biological, and functional properties of lost or damaged dental tissues are rarely restored. Tissue engineering offers a promising alternative by integrating scaffolds, cells, and signaling molecules to regenerate functional dental tissues. A critical component of this strategy is the development of bioactive scaffolds that not only support cell adhesion and proliferation but also actively guide stem cell differentiation toward odontogenic lineages and promote mineralized extracellular matrix (ECM) deposition.[1,2]

Over the past decade, scaffold-based strategies in dental tissue engineering have progressed from simple biodegradable polymers to advanced biomimetic composite systems designed to replicate the native extracellular microenvironment. Among the various biomaterials investigated, natural polymers such as chitosan and gelatin have attracted considerable interest due to their excellent biocompatibility, biodegradability, and structural similarity to native ECM components. Chitosan, a polysaccharide derived from chitin, exhibits antibacterial and hemostatic properties and supports cell adhesion and proliferation. Its cationic nature enables electrostatic interactions with negatively charged cell membranes and biomolecules, enhancing cellular attachment and

bioactivity. Gelatin, a denatured form of collagen, contains bioactive Arg-Gly-Asp (RGD) sequences that facilitate integrin-mediated cell adhesion, spreading, and migration.[3,4]

Despite these biological advantages, chitosan and gelatin suffer from inherent limitations when used alone for hard tissue regeneration. These include poor mechanical strength, rapid degradation, and limited ability to support mineralization, which restrict their application in dentin and bone tissue engineering.[5] Consequently, incorporation of inorganic bioactive components has been explored to enhance their structural and functional properties. Among these, nanohydroxyapatite (nHAp) has emerged as a particularly promising reinforcement material. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is the primary inorganic constituent of bone and dentin, and its nanoscale form exhibits a high surface area-to-volume ratio, enhanced bioactivity, and improved interfacial bonding with polymer matrices. nHAp improves the mechanical stability of composite scaffolds while providing favorable sites for protein adsorption, ion exchange, and mineral nucleation, all of which are essential for osteogenic and odontogenic differentiation.[6,7]

The compositional and structural similarity of nHAp to native dental hard tissues makes it particularly suitable for dental tissue engineering applications. Multiple studies have demonstrated that nHAp-containing scaffolds enhance odontogenic differentiation of dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), and other mesenchymal stem cells (MSCs). This effect is evidenced by increased alkaline phosphatase activity, upregulated expression of odontogenic markers, and enhanced mineral deposition.[8–10] Building on these findings, hybrid scaffold systems combining chitosan, gelatin, and nanohydroxyapatite (CS/Gel/nHAp) have been developed to synergistically integrate the biological benefits of natural polymers with the mechanical strength and bioactivity of ceramic components.

CS/Gel/nHAp hybrid scaffolds are designed to mimic the biochemical composition, structural organization, and mechanical properties of the native dentin–pulp complex. In these systems, chitosan provides antimicrobial activity and structural integrity, gelatin enhances cell adhesion through bioactive motifs, and nHAp contributes mechanical reinforcement and mineralization cues. Together, these components create a biomimetic microenvironment conducive to stem cell survival, proliferation, odontogenic differentiation, and mineralized tissue formation. Several in vitro investigations have reported increased expression of odontoblastic and osteogenic markers—including dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP1), alkaline phosphatase (ALP), and runt-related transcription factor-2 (RUNX2)—in cells cultured on CS/Gel/nHAp scaffolds. These molecular findings are often accompanied by enhanced calcium deposition, mineral nodule formation, and positive alizarin red and von Kossa staining, indicating robust in vitro biomineralization.[11–13]

Beyond material composition, scaffold physicochemical properties such as porosity, pore size distribution, surface roughness, mechanical stiffness, and degradation rate play critical roles in regulating cellular responses and mineralization outcomes. High porosity and interconnected pore networks facilitate cell migration and nutrient diffusion, while scaffold stiffness influences stem cell fate through mechano transduction pathways. Controlled degradation rates are essential to ensure gradual replacement of the scaffold with newly formed tissue. Importantly, these properties are strongly influenced by scaffold fabrication techniques, including freeze-drying, electrospinning, solvent casting, and three-dimensional (3D) printing, each of which produces scaffolds with distinct microarchitectural characteristics.

Despite the increasing number of studies investigating CS/Gel/nHAp scaffolds for dental tissue engineering, the existing evidence remains fragmented and heterogeneous. Significant variability exists among studies with respect to scaffold composition, material ratios, fabrication methods, crosslinking strategies, cell sources, culture conditions, and outcome assessment techniques. This heterogeneity complicates direct comparison of results and limits the ability to identify optimal scaffold formulations. Furthermore, although enhanced odontogenic differentiation and mineralization are frequently reported, the molecular mechanisms through which individual scaffold components influence odontogenic signaling pathways remain incompletely understood.[14–16]

Systematic reviews provide a structured approach for synthesizing and critically evaluating available evidence, enabling identification of consistent trends, methodological limitations, and gaps in knowledge. A focused systematic review of CS/Gel/nHAp scaffolds used in dental tissue engineering—particularly studies evaluating odontogenic differentiation and in vitro biomineralization—can offer valuable insights into how scaffold composition, fabrication techniques, and structural properties influence biological performance. Such synthesis is essential for establishing evidence-based design principles for biomimetic scaffolds intended for dentin–pulp regeneration.

The rationale for conducting this systematic review is

further strengthened by the translational potential of CS/Gel/nHAp scaffolds in regenerative endodontics and restorative dentistry. In addition to biological efficacy, successful clinical translation requires scaffold systems that are reproducible, scalable, and compatible with regulatory requirements. Material selection, fabrication methods, and component ratios can significantly influence manufacturability and regulatory approval pathways. Therefore, consolidating current evidence is crucial for guiding both experimental research and future clinical applications.

Accordingly, the primary objective of this systematic review is to critically evaluate and synthesize experimental evidence on hybrid chitosan/gelatin/nanohydroxyapatite scaffolds with respect to their ability to promote odontogenic differentiation of stem cells, as assessed by gene and protein expression markers such as DSPP, DMP1, ALP, and RUNX2. Secondary objectives include evaluating in vitro biomineralization outcomes using calcium deposition assays, alizarin red staining, von Kossa staining, and related analyses, identifying scaffold properties associated with enhanced biological performance, and comparing fabrication techniques and material ratios to determine optimal scaffold designs for dental tissue engineering. By achieving these aims, this review seeks to provide a comprehensive and evidence-based overview of CS/Gel/nHAp scaffold research and to support the advancement of biomimetic strategies in regenerative dentistry.

MATERIALS AND METHODS

This systematic review was conducted and reported in accordance with the PRISMA guidelines.

Focused question

How do hybrid chitosan/gelatin/nanohydroxyapatite scaffolds influence odontogenic differentiation of dental pulp stem cells and in vitro biomineralization?

Eligibility criteria (PICOS)

Population: Dental pulp stem cells or mesenchymal stem cells

Intervention: CS/Gel/nHAp hybrid scaffolds

Comparison: Cells without scaffolds or alternative scaffold materials

Outcomes: Odontogenic differentiation markers and in vitro biomineralization

Study design: In vitro human and animal studies

Information sources and search strategy

Electronic searches were performed in PubMed, Scopus, ScienceDirect and Google Scholar for studies published in English up to April 2024. Search terms included combinations of "chitosan", "gelatin", "nanohydroxyapatite", "odontogenic differentiation" and "dental pulp stem cells".

Study selection

The electronic database search identified 312 records. After removal of 78 duplicates, 234 records were screened by title and abstract. Of these, 198 records were excluded for not meeting the inclusion criteria. Thirty-six full-text articles were assessed for eligibility, of which 29 were excluded due to use of non-hybrid scaffolds, absence of odontogenic outcomes or non-relevant study design. Finally, seven studies fulfilled the predefined inclusion criteria and were included in the qualitative synthesis. The study selection process is illustrated in the PRISMA flow diagram (Figure 1).

Study characteristics

The main characteristics of the included studies, including scaffold composition, cell source, experimental design and primary outcomes, are summarised in Table 1.

RESULTS

Seven studies were included in the qualitative synthesis. The included studies demonstrated consistent improvements in cell viability, odontogenic gene expression and mineralized matrix formation when CS/Gel/nHAp scaffolds were used.

Odontogenic differentiation

Expression of odontogenic markers including DSPP, DMP1, ALP and RUNX2 was significantly higher in cells cultured on CS/Gel/nHAp scaffolds compared with controls.

In vitro biomineralization

Enhanced calcium deposition and mineralized nodule formation were reported across studies, confirming the biomineralization potential of the hybrid scaffolds.

DISCUSSION

This systematic review evaluated the effectiveness of hybrid chitosan/gelatin/nanohydroxyapatite (CS/Gel/nHAp) scaffolds in promoting odontogenic differentiation and in vitro biomineralization. The synthesis of current evidence indicates that these tri-component composite scaffolds possess substantial potential for dental tissue engineering, particularly in dentin–pulp regeneration and related applications.

The integration of chitosan, gelatin, and nanohydroxyapatite leverages the complementary advantages of each component while mitigating their individual limitations. Chitosan offers excellent biocompatibility, biodegradability, and antimicrobial

activity but exhibits limited mechanical strength and bioactivity when used alone. Gelatin, derived from collagen, provides Arg-Gly-Asp (RGD) motifs that support cell adhesion, proliferation, and migration; however, its rapid degradation and poor mechanical stability restrict its independent use in hard tissue regeneration. Nanohydroxyapatite, which closely resembles the inorganic phase of bone and dentin, enhances osteo/odonto-conductivity and significantly improves scaffold mechanical properties.[16] The synergistic combination of these materials results in a biomimetic microenvironment that closely replicates the native dentin–pulp extracellular matrix (ECM), thereby facilitating cellular activities essential for tissue regeneration.

Across the included studies, CS/Gel/nHAp scaffolds consistently demonstrated improved stem cell attachment, proliferation, and differentiation—key prerequisites for successful dentin–pulp regeneration.[17] Odontogenic differentiation, a central objective in dental regenerative therapies, was markedly enhanced on these hybrid scaffolds. The reviewed literature consistently reported upregulation of critical odontogenic markers, including dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP1), alkaline phosphatase (ALP), and runt-related transcription factor-2 (RUNX2).[18,19]

Nanohydroxyapatite played a pivotal role in this biological response. Beyond serving as a mechanical reinforcement, nHAp acts as a bioactive signaling component capable of influencing intracellular calcium homeostasis and activating gene expression pathways associated with mineralized tissue formation. Studies employing dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), and mesenchymal stem cells (MSCs) reported significantly higher ALP activity and calcium deposition when cultured on CS/Gel/nHAp scaffolds compared with control materials. These findings suggest that the scaffold not only supports cell viability but actively promotes odontogenic lineage commitment.[20–22]

In vitro biomineralization outcomes were also consistently superior in CS/Gel/nHAp scaffolds. Enhanced mineralized nodule formation, confirmed by alizarin red S staining, von Kossa staining, and calcium quantification assays, was observed across multiple studies. Mineral deposition was more abundant and uniformly distributed than in scaffolds lacking one or more components. This enhanced biomineralization can be attributed to the bioactive nature of nHAp, which provides nucleation sites for calcium phosphate crystallization, as well as optimized scaffold porosity and surface roughness that facilitate nutrient diffusion and cell–matrix interactions.[23,24] Interconnected porous networks further supported homogeneous mineral distribution throughout the scaffold.

Scaffold composition and fabrication methods were identified as critical determinants of biological performance. Scaffolds containing moderate nHAp concentrations (typically 20–40% w/w) exhibited optimal mineralization and mechanical properties. However, excessive ceramic content resulted in increased brittleness and reduced cell viability, underscoring the importance of balancing scaffold composition. Among fabrication techniques, freeze-drying (lyophilization) was most commonly associated with favorable outcomes, owing to its ability to generate highly porous, interconnected structures with pore sizes ranging from 100–300 µm—dimensions considered optimal for cell infiltration and vascularization. Although three-dimensional (3D) printing allows precise control over scaffold geometry, its application in CS/Gel/nHAp systems remains limited. Cross-linking agents such as glutaraldehyde and genipin were employed to enhance mechanical stability and control degradation rates; however, concerns regarding cytotoxicity necessitate careful optimization.[25,26]

Mechanical and degradation properties were also improved by nHAp incorporation. The reviewed studies demonstrated increased compressive strength and modulus, bringing scaffold properties closer to those of native dentin. Nevertheless, excessive nHAp loading or insufficient cross-linking produced brittle scaffolds unsuitable for load-bearing conditions. Scaffold biodegradability emerged as another critical factor, as ideal scaffolds should degrade in synchrony with new tissue formation. While chitosan and gelatin undergo enzymatic degradation, nHAp incorporation generally slowed degradation rates, potentially supporting prolonged regenerative processes. Precise control of degradation kinetics, however, remains a challenge and warrants further investigation.[27–29]

Compared with mono-component or biphasic scaffolds, CS/Gel/nHAp systems consistently outperformed alternatives in promoting odontogenic differentiation and biomineralization. Although synthetic polymers such as polycaprolactone (PCL), polylactic acid (PLA), and PLGA have been explored for dental applications, their limited intrinsic bioactivity often necessitates surface modification or biofunctionalization. In contrast, the natural origin and inherent bioactivity of CS/Gel/nHAp scaffolds make them particularly attractive for regenerative endodontics and dentin–pulp complex reconstruction.[30,31]

Limitations of the Study

Despite encouraging findings, several limitations were identified across the included studies:

1. Lack of standardized protocols for scaffold composition, fabrication, and

characterization hindered direct comparison between studies.
2. Small sample sizes and short-term in vitro culture periods limited the generalizability of results.
3. Variability in outcome assessment methods, including differences in odontogenic markers and ALP assay time points, reduced data uniformity.
4. Limited in vivo validation, with most studies restricted to in vitro models and only a few incorporating animal experiments to assess functional integration.

Future Recommendations

Based on the current evidence and identified gaps, future research should focus on:

1. Standardizing scaffold fabrication, characterization, and biological evaluation protocols to enhance reproducibility and comparability.
2. Conducting long-term in vitro and in vivo studies to assess the durability of odontogenic differentiation and mineralization.
3. Incorporating bioactive molecules such as growth factors (e.g., BMP-2, TGF-β1, FGF) to further enhance odontogenic signaling.
4. Utilizing advanced fabrication approaches, including 3D bioprinting and microfluidic platforms, to create physiologically relevant dentin–pulp models.
5. Evaluating immunogenicity and inflammatory responses, particularly in large-animal models, to support clinical translation.

CONCLUSION

In conclusion, this systematic review demonstrates that hybrid chitosan/gelatin/nanohydroxyapatite scaffolds exhibit significant potential for promoting odontogenic differentiation and in vitro biomineralization. The synergistic interaction of the tri-component system enhances both biological and mechanical scaffold performance, positioning CS/Gel/nHAp scaffolds as promising candidates for future dental regenerative applications. Nevertheless, further preclinical and clinical investigations are required to validate these findings and facilitate translation into clinical practice.

PRISMA flow diagram

Figure 1: PRISMA Flow chart presenting the screening process

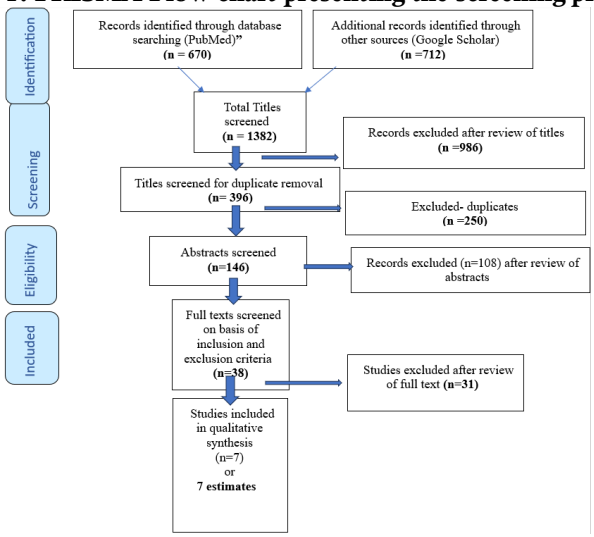

Table 1. Characteristics of the included studies evaluating CS/Gel/nHAp scaffolds for odontogenic differentiation and biomineralization.

Author (year)	Study type	Cell source	Scaffold composition	Outcomes assessed	Main findings
Basdra et al. (2020)	In vitro	Human dental pulp stem cells	CS/Gel/nHAp	DSPP, DMP1, ALP, mineralization	Significant enhancement of odontogenic differentiation and mineralized matrix formation
Peter et al. (2010)	In vitro	Osteoblast-like cells	CS/Gel/nHAp	Cell adhesion, proliferation	Improved biocompatibility and scaffold stability
Wu et al.	In vitro	Periodontal	nHAp/CS/Gel	Osteogenic	Enhanced bone and mineral

Author (year)	Study type	Cell source	Scaffold composition	Outcomes assessed	Main findings
(2021)	/ in vivo	ligament stem cells		markers, mineralization	regeneration
Kooti et al. (2024)	In vitro	Mesenchymal stem cells	CS/Gel/nHAp composite	Cell viability, mineralization	Increased bioactivity and osteo/odontogenic potential
Qu & Liu (2013)	In vitro	Human dental pulp stem cells	Gelatin-based hybrid scaffold	Odontogenic differentiation	Upregulation of odontogenic markers
Shalumon et al. (2018)	In vitro	Stem cells	Gelatin/nHAp hybrid	Mineral deposition	Improved biomineralization
Feng et al. (2014)	In vitro	Dental pulp stem cells	Chitosan scaffold	Neural and odontogenic differentiation	Enhanced stem cell survival and differentiation

Conflict of interest

The authors declare no conflicts of interest.

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