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+The Osteo-inductive and Osteo-conductive Potential of Nano-Chitosan-Based Composites in Bone Tissue Regeneration: A Systematic Review of In-vitro Studies Analysed by Quinn Tool

Dr. Karuppiah Gokulapriyan[1*], Dr. Ramya Ramadoss[2*], Dr. Pavithra R[3], Dr. Shekar Shobana[4], Dr. A. Shafie Ahamed[5], Dr. Adhithiya Palaniswamy[6], Dr. Jaganiya Sekar[7], Dr. Mayuri Rapeti[8], Dr. Hema Shree K[9]

[1] *Research Scholar, Saveetha University, Associate Professor, Department of Conservative Dentistry and Endodontics, Best Dental Science College, Madurai, Tamil Nadu Dr. MGR Medical University, Chennai, Tamil Nadu, India*
[2] *Professor and Head, Department of Oral Biology, Saveetha Dental College, Saveetha University, Chennai, Tamil Nadu, India*
[3] *Associate Professor, Department of Public Health Dentistry, Sri Venkateswara Dental College & Hospital, Chennai, Tamil Nadu, India*
[4] *Associate Professor, Department of Conservative Dentistry and Endodontics, Tagore Dental College, Tamilnadu Dr. MGR Medical University, Chennai, Tamil Nadu, India*
[5] *Professor and Head, Department of Conservative Dentistry and Endodontics, Government Dental College, Cuddalore, Tamil Nadu, India*
[6] *DMD Student, Rutgers School of Dental Medicine, 110 Bergan Street, Newark, NJ07103*
[7] *Dental Assistant, London, Ontario, Canada*
[8] *Dental Assistant, OH State, ZIP 45040*
[9] *Research Scientist, Department of Oral Biology &Oral Pathology, Saveetha Dental College & Hospitals, Chennai, Tamil Nadu, India*
**Corresponding Author: Dr. Karuppiah Gokulapriyan, Dr. Ramya Ramadoss*

KEYWORDS

bone, chitosan, chelation, demineralization, fibroblast, nanoparticle, nano-chitosan, osteo-conduction, osteocytes, osteo-induction, remineralization

ABSTRACT

Background: Chitosan-based composites have gained significant attention in bone tissue engineering due to their biocompatibility, biodegradability, and intrinsic bioactivity. These materials are increasingly explored for their potential to enhance bone regeneration, particularly through their osteoconductive and osteoinductive properties. This systematic review aims to critically assess the current evidence on the effectiveness of nano chitosan-based composites in promoting bone tissue regeneration.

Methodology: A comprehensive literature search was performed across multiple electronic databases, including PubMed, Google Scholar, the Trip Medical Database, and EBSCO. The search was conducted from November 2023 to July 2024, and it includes studies published till November 2023. The search terms were carefully selected using the MeSH Browser and tailored for each database. Inclusion criteria were focused on in vitro studies investigating the osteoconductive and osteoinductive properties of chitosan-based composites in bone regeneration. Systematic reviews, meta-analyses, and in vivo studies that did not specifically address the role of chitosan in bone healing were excluded. The quality and risk of bias of the included studies were assessed using the QUINN assessment tool.

Results: Twelve studies met the inclusion criteria and were included in the qualitative analysis. These studies explored various nano-chitosan-based composites, often combined with osteoconductive materials like hydroxyapatite or bioactive glass, etc. The findings across these studies consistently demonstrated that chitosan-based composites enhance osteogenic differentiation of precursor cells and promote bone matrix deposition. Additionally, the osteoinductive capacity of these composites were evident by their ability to induce mesenchymal stem cell differentiation into osteoblasts and facilitate new bone formation in animal models. However, the methodological quality of the included studies varied, with most studies exhibiting a moderate risk of bias. The key challenges identified included issues with randomization, blinding, and sample size calculations, which may affect the generalizability of the findings. Despite these limitations, the results suggest that nano chitosan-based composites hold significant potential as biomaterials for bone tissue regeneration.

Conclusion: This systematic review underscores the potential of chitosan-based composites in bone tissue engineering, particularly for their osteoconductive and osteoinductive properties. While the current evidence is promising, further high-quality research is necessary to confirm these findings and facilitate the translation of these materials into clinical practice. Addressing the methodological limitations identified in this review will be crucial for advancing the field and improving patient outcomes in bone regeneration therapies.

1. Introduction

Bone makes up the human skeleton, which contains connective tissue and specialized cells that deposits and break down calcium. Bone is formed by deposition of calcium by osteoblast and osteocyte cells. Likewise, osteoclast cells breakdown the tissue in bones and release minerals, resulting in a transfer of calcium ions from the bone tissue to the blood[1]. Osteoclast[2,3] are multinucleated giant cells that differentiate, when in contact with M-CSF (macrophage colony - stimulating factor) and RANKL[4] (receptor activator of NF-κB ligand), which are presented by osteoblast and osteocytes.[5,6,7] Bone remodelling[8,9,10] is a process which maintains bone strength and homeostasis by replacing discreet part of old bone with newly synthesised packets of proteinaceous matrix. Osteoclast and osteoblast maintain this skeletal integrity throughout life by a process termed as osteoclast-osteoblast coupling.[5,6,7] In case of tooth dentin[11], it is replaced by odontoblast as tertiary dentin[12], a similar process of mineralisation compared to bone. On the other hand, demineralisation- a process of removal of calcium and phosphate ions from bone due to trauma or infection may also lead to resorption.[13,14]

Resorption[14] is a spontaneous process that occurs in the body. It is affected by physiological as well as pathological in terms of traumatic injury or infection respectively. Treating and replacing these affected zones of bony defect in time, is essential in certain cases whenever spontaneous healing is difficult. Over the years, surgical interventions such as removal of defective bone, bone graft placement, bone augmentation, or bone implants or plates etc, have been considered to treat bony defects.[15] Although these methods, however, have disadvantages, including erratic bone regrowth, donor site morbidity, foreign body reactivity, and variable absorption rate. A tissue engineering[16,17] strategy that combines osteogenic stem cells with an appropriate scaffolding material shall be recommended for the healing of bone defects.

There is strenuous search of a new material or drug that can facilitate and aid in the healing of bone effortlessly and with minimum or no complications. In the recent years the research on chitosan a naturally occurring glycosamide bio-polymer, second only to cellulose, have gained focus due to its abundant availability, biocompatibility, non-toxic, antimicrobial, chelating, muco-adhesiveness, drug delivering ability and many others. Nano-Chitosan is one such material that is a boon in the field of orthopaedics and dentistry in terms of bone healing.[18,19,20,21]

The number of research studies on chitosan in the recent years has increased with over 2500 publication per year in the last decade. Although there is significant research investigations published, very few literatures that support the bone repair and osteogenic property of chitosan are reported. And hence a need for a systematic review on chitosan's ability to induce and osteo-conduction over bone is necessary. The hypothesis taken is chitosan has osteoconductive and osteo-inductive effect on bone repair.

2. Materials and Methods

Research question: Does Nano-Chitosan has osteo-conductive and osteo-inductive property on bone?

2.1. Literature search strategy:

The review was carried out between November 2023 and July 2024, focussing on studies published in the last 10years from 2013 to 2023. Two reviewers used the electronic databases such as Pubmed, Google Scholar, Trip medical database, and Ebsco to conduct a thorough literature search until November 2023 for papers evaluating the osteo-conductive and osteo-inductive properties of nano-chitosan on bone in accordance with particular search keywords (Table 1). Bibliographies of earlier reviews on the topic and all publications cited in these articles were also found through additional manual search.

2.2. Search terms:

MeSH Browser was accessed to identify entry terms and compose the final Boolean searches. The

following search terms or equivalent were used: bone, chitosan, chelation, demineralisation, fibroblast, osteo-conduction, nanoparticle, nano chitosan, osteocytes, osteo-induction, remineralisation. The search terms were adapted for each database.

2.3. Eligibility criteria: Studies that focused on the action of chitosan on bone healing were included for the review. Only in-vitro studies published in journals were included. Articles published in English alone were included, and those with English translation provided for other languages.

2.4. Inclusion and Exclusion criteria: The review focused on studies investigating the **osteoconductive and osteo-inductive properties of chitosan-based composites**. Only **in vitro** studies published in English were considered. Studies that focused on chitosan's role in bone healing, whether alone or in combination with other bio-materials, were included. In-vivo and/ clinical studies were excluded. **Systematic reviews and meta-analyses** that did not specifically address chitosan's osteogenic potential were excluded to maintain a focused analysis.

2.5 Study selection: Abstracts of all selected papers were evaluated by two evaluators independently. When information present in the title and abstract was insufficient, the full text version was retrieved for further assessment. A comparison of 12 different searches was carried out to delete the repeated entries. Full text format was obtained for all the articles that met with the inclusion and exclusion criteria. The consort flow diagram for selection of studies is given in flowchart 1.

2.6. Data extraction: Microsoft Excel was used to build a data extraction form, and two separate reviewers took the data out of each of the chosen papers. A third reviewer was consulted in order to resolve disagreements amongst the reviewers. The following information was included in the data extraction form: author and year, design, abstract, values, and conclusion together with the measured parameters. Microsoft Excel version 2021 was used to tabulate data that was taken out of each article. (Table 3)[22-33]

2.6. Quality and risk of bias assessment: Utilizing the QUINN[34] evaluation tool to gauge the risk of bias for in-VITRO research, two impartial reviewers evaluated the quality of the included papers. Every evaluation was completed on an individual basis. The Quinn tool mentions twelve criteria for the assessments: clearly defined goals and objectives; a thorough explanation of sample size calculation; sampling technique; comparison group details; an explanation of methodology; operator details; randomization; methods of measuring outcome; outcome assessor details; blinding; statistical analysis; and presentation of details. Out of the twelve criteria four criteria such as sample size calculation, sampling technique, randomization and blinding were considered as not applicable and only remaining 8 criteria were used for grading the individual study scoring.

Each of the selected studies were scored as follows; (a) adequately specified =2, (b) inadequately specified, (c) not specified, (d) Not applicable. The score was then added to obtain a total score for a particular study and formula given below

$$\textbf{Formula:} \quad \textbf{final score} = \frac{\textit{total score X } 100}{2 \textit{ X No.of criteria applicable}}$$

was used to grade individual studies as high risk (>50%), medium risk (50-70%) and low risk (>70%) (Table 2). An expert in the subject arbitrated disputes that arose during this assessment procedure.

3. Results

A total of 12 studies met the inclusion criteria and were included in the qualitative analysis. The quality of the included studies was moderate, with most studies demonstrating a medium risk of bias. Nano-Chitosan-based composites, particularly in combination with osteoconductive materials, were consistently shown to promote osteogenic differentiation of precursor cells, enhance bone matrix deposition, and support new bone formation.

3.1 Characteristics of Studies:

The 12 studies included in this review explored a wide range of chitosan-based composites for bone tissue regeneration, typically combined with osteoconductive agents like hydroxyapatite, calcium phosphate, and bioactive glass. Most studies were in vitro, focusing on assessing the biological properties of chitosan composites, such as cell viability, proliferation, osteogenic differentiation, and the expression of osteogenic markers like alkaline phosphatase and collagen I.

3.2 Key parameters evaluated in these studies included:

- **Cell Viability and Proliferation**: Various assays such as Alamar Blue and MTT assay{3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} were used to determine cell viability and growth on chitosan-based scaffolds.
- **Osteogenic Differentiation**: Markers like alkaline phosphatase activity and osteocalcin expression were commonly assessed.
- **Mechanical Properties**: Studies measured parameters such as compressive strength, modulus of elasticity, and scaffold porosity to optimize structural properties for bone tissue engineering.
- **Composite Structure**: Chitosan was often combined with osteoconductive materials, with several studies emphasizing the synergistic effects of these composites on enhancing bone regeneration.

3.3 Risk of Bias Assessment

The risk of bias in the selected studies was assessed using the **QUINN evaluation tool**[34], with scores assigned based on 8 out of 12 applicable criteria. Studies were categorized as high, medium, or low risk of bias according to their final scores. A total of **four** studies were rated as having a **high risk of bias**, as they scored below 50% on the assessment, while the remaining eight studies exhibited a **medium risk of bias**, with scores ranging between 50% and 70%.

Flowchart 1: Consort Flow Diagram

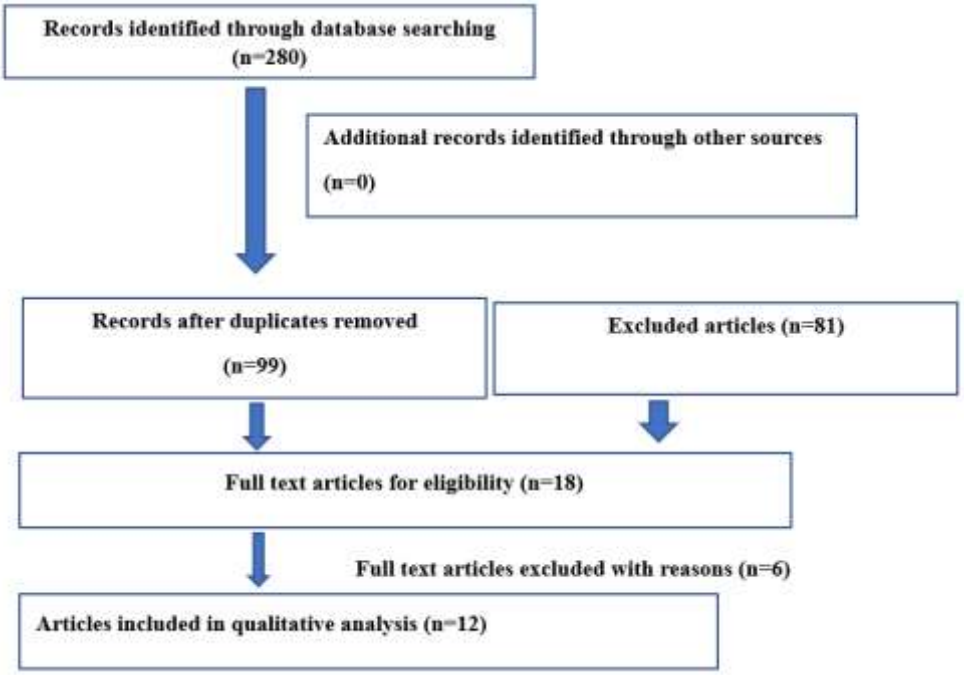

Table 1: Keywords Used for Search and Number of Articles Retrieved from Database

Pubmed	Tripmedical	Ebsco	Google scholar
229	1	0	50
Keywords	bone, chitosan, chelation, demineralisation, fibroblast, nanoparticle, nano-chitosan, osteo-conduction, osteocytes, osteo-induction, remineralisation		

Table 2: Assessment of Risk of Bias using Quinn Tool for In-Vitro Studies

Sno	Criteria	K zafeiris 2020	AitorTejo 2021	Mahboubeh Rezazadeh 2018	Gentao Qiu 2020	Piotr Kowalczyk 2021	Paulina Kazimierczak 2021	Yamini Chandramohan 2020	Fatemeh Hajezi 2020	ShixhouWu 2020	Mohammad Porgham Daryasari 2019	Premjit 2020	Agata Przekora 2021
1	Clearly stated aims and objectives	2	1	1	2	1	2	1	2	2	1	2	1
2	Details of comparision group	1	1	1	1	1	1	1	1	1	1	1	1
3	Detailed explanation of methodology	1	1	1	1	1	1	1	1	1	1	1	1
4	Operator details	0	0	0	0	0	0	0	0	0	0	0	0
5	Methods of measurement of outcome	1	1	1	1	1	1	1	1	1	1	1	1
6	Outcome assessor details	0	0	0	0	0	0	0	0	0	0	0	0
7	Statistical analysis	2	2	2	2	2	1	2	2	1	2	2	1
8	Presentation of details	2	2	2	1	1	1	2	2	1	1	2	1
Total score		9	8	8	8	7	7	8	9	7	7	9	6
Risk of bias		Medium	Medium risk	Medium risk	Medium risk	High risk	Medium risk	Medium risk	Medium risk	High risk	High risk	Medium risk	High risk

Table 3: Table of Characteristics

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
1	K. Zafeiris, D. Brasinika[22] 2020	IN VITRO	The article discusses the additive manufacturing of hydroxyapatite-Chitosan-Genipin composite scaffolds for bone tissue engineering applications	1.The study utilized data on ink flow speed, porosity percentages, and pore size distribution to optimize the printing process and enhance scaffold properties. 2. Mechanical properties enhancement and cell viability data were analyzed to assess the effectiveness of the developed interconnected porous network for bone tissue engineering. 3. Printing trials involved adjusting parameters like nozzle tip diameter, flow speed, infill, and perimeter speed to achieve the desired 3D structure and scaffold microarchitecture	1.Among 0.58,0.41 and 025mm nozzles; 0.41mm nozzle were selected optimum. 2.All samples presented modulus higher than 0.6 GPa, which falls within the corresponding values of the natural cancellous bone ranging between 0.1 GPa and 2 GPa[53,54]. Hardness values were estimated between 0.12 GPa and 0.25 GPa.	The 3D printed scaffolds of HAP-Chitosan-Genipin-L-arginine, mimic the extracellular matrix and ideally serve as bone substitutes, promoting bone regeneration. It showed cytocompatibility and had decreased porosity of 3D scaffold, and had increased developed interconnected porous network promising for osteogenesis.
2	Aitor Tejo-Otero and Alastair Ritchie[23]/ 2021	In vitro	Chitosan and gelatin are commonly used in tissue engineering due to their biocompatibility, but their poor mechanical properties limit their use in bone tissue engineering. □ By adding calcium minerals to the hydrogel mix, both mechanical and biological properties of the scaffolds were improved, with higher mineral content leading to better mechanical properties and enhanced cell proliferation and mineralization[	1. The absorbance of the Alamar Blue Assay was used to indicate cell proliferation and viability. 2.Mechanical properties were evaluated by measuring compressive load-deformation curves and Young's Modulus 3.Statistical analysis was performed using MATLAB to compare different concentrations at a single time point. 4. Cell proliferation and mineralization were assessed through biological tests using Alamar Blue and Alizarin Red Assay	In both cell proliferation and mineralization, the following compositions are the best: 50% CaCO3/50% CaHPO4 and 80% CaCO3/20% CaHPO4 for 80% Hydrogel/20% Minerals and 70% Hydrogel/30% Minerals mixtures, respectively. On the other hand, the mechanical properties were	1.The addition of calcium compounds to hydrogels resulted in higher cell proliferation and better mineralization. 2. Optimal compositions for biological properties were found to be 50% CaCO3 and 50% CaHPO4, and 80% CaCO3 and 20% CaHPO4 for different hydrogel mixtures. 3.Mechanical properties were enhanced with 100% CaCO3 in both hydrogel mixtures. 4.Mineralized groups showed better mineralization compared to

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
					better at 100% CaCO3 for both mixtures	demineralized groups, indicating the importance of mineralization for cell proliferation and osteogenic differentiation
3	Mahboubeh Rezazadeh et al[24] 2018	In vitro	□Development of a localized controlled delivery system for Rosuvastatin (RSV) using chitosan/chondroitin sulfate nanoparticles in a thermosensitive hydrogel showed improved osteoblast viability and proliferation. □Optimization of RSV-loaded nanoparticles embedded in a hydrogel composed of hyaluronic acid and Pluronic F127 demonstrated controlled release of RSV and enhanced cellular behavior for bone tissue engineering	1.Factors included CTS solution concentration, pH of CTS solution, and CTSCS ratio in a 3-level Box-Behnken design. □Variables assessed were particle size, zeta potential, entrapment efficiency, and mean release time of the optimized RSV-loaded nanoparticles. □The study evaluated the impact of various formulation variables on drug loading, zeta potential, entrapment efficiency, and mean release time of the nanoparticles. □Particle size ranged from 226 to 996 nm for different factor level combinations, with a quadratic equation explaining the effect of each factor on particle size. □Mean release times (MRTs) ranging from 3.09 to 8.33 hours were analyzed, with a regression equation describing the effect of each factor on MRT values.	Mean particle size, zeta potential, entrapment efficiency, and mean release time of the optimized RSV-loaded nanoparticles were confirmed as 283.2 ± 16 nm, –31.2 ± 6.8 mV, 63.1±4.2%, and 6.14± 0.3 h respectively Hydrogel engrafted with CTS/CS showed controlled release of RSV during 48 h with superior in vitro gel stability.	1. the hydrogel of RSV-loaded CTS/CS nanoparticles exhibited sustained release of RSV during 48hrs & could maintain osteoblast osteoblast viability and proliferation Promising for bone tissue engineering
4	Gengtao Qiu[25]/2021	In vitro	□A novel antibacterial scaffold of chitosan-reinforced calcium phosphate cement delivering doxycycline hyclate (CPCC + DOX) was developed for bone regeneration and infection control. □□The CPCC + DOX scaffold exhibited strong antibacterial effects against S. aureus and P. gingivalis, with enhanced bone regeneration potential when	1.Antibacterial effects against S. aureus and P. gingivalis 2.Flexural strength of CPCC + DOX scaffolds 3.DOX release from scaffolds over 21 days 4.Viability of hPDLSCs encapsulated in alginate beads during culture with CPCC + DOX scaffolds 5.Alkaline phosphatase activity, mineral synthesis,	□ CPCC + DOX5mg scaffold had strong antibacterial effect against S. aureus. □ CPCC + DOX5mg group showed higher osteogenic gene expressions	1.The novel CPCC + DOX scaffold demonstrated strong antibacterial effects against S. aureus and P. gingivalis, making it a promising candidate for treating bone defects . 2. scaffold of hPDLSCs with CPCC+Dox successfully differentiated to osteogenic lineage to

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
			combined with human periodontal ligament stem cells encapsulated in alginate beads	and osteogenic gene expressions in hPDLSCs	than control. □ hPDLSCs from microbeads had high viability in coculture with CPC. □Encapsulated hPDLSCs released from microbeads and proliferated rapidly.	support cell growth and viability.
5	Piotr Kowalczyk[26]/2021	In vitro	1.Chitosan-human bone composite granulates were developed for guided bone regeneration, showing non-cytotoxicity, suitability for cell growth, and increased alkaline phosphatase activity. 2.The granulate composition included β-tricalcium phosphate, pulverized human bone, and chitosan, prepared using an encapsulator and suitable for thermal sterilization	□Granules were prepared in triplicates to ensure repeatability of the process. □Size distributions were analyzed for collective particle counts, mean sizes, and modes of size for each repetition of granulate preparation. □Particle area was determined using ImageJ software, with only particles meeting specific criteria processed to avoid counting artifacts. □Granules were characterized using scanning electron microscopy (SEM) to visualize the samples and investigate the presence of functional groups on the surfaces of the materials. □Fourier-transform infrared spectroscopy (FTIR) was used to analyze the presence of functional groups on the surfaces of the materials		□The composite β - TCP/human bone composites (chitosan-calcium phosphate-human bone granulate) showed non-cytotoxicity, cell viability, and increased alkaline phosphatase activity, making it suitable for guided bone regeneration than β - TCP composites. □The material can be easily prepared and thermally sterilized with an autoclave, showing potential for regenerative applications
6	Paulina Kazimierczak[27]/2021	In vitro	□The chitosan/agarose/nanoHA bone scaffold induces M2 macrophage polarization, releasing anti-inflammatory cytokines, and enhancing osteogenic differentiation. □Macrophages cultured on the scaffold predominantly exhibit the M2 phenotype,	1.Mono-culture experiment for macrophage characterisation evaluated the level of (IL-1β, IL-6, TNF-α) pro-inflammatory and (IL-4,IL-10, IL-3, TGF-β1) anti-inflammatory factors in cell culture supernatants. 2.effect of M2 macrophages	The level of osteogenic markers in BMDSCs and normal human fetal osteoblasts (hFOB1.19)	□The chitosan/agarose/nanoHA bone scaffold induced M2 macrophage polarization, releasing anti-inflammatory cytokines and enhancing osteogenic differentiation.

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
			with low levels of proinflammatory cytokines, indicating a low risk of inflammatory response	on osteogenic differentiation in co-culture system		□ M2 macrophages promoted the production of osteogenic markers in stem cells, such as collagen I and osteocalcin, indicating the positive effect of macrophages on osteogenic differentiation
7	Yamini Chandramohan[28]/2020	IN VITRO	1.The study focuses on utilizing human ovarian follicular fluid-derived mesenchymal stem cells in a chitosan/PCL/Zn scaffold for bone tissue regeneration. 2.The research involves characterizing the MSCs from ovarian follicular fluid, assessing their stemness, proliferation, and differentiation potential, and testing their suitability for bone tissue engineering applications	1.The study utilized human ovarian follicular fluid-derived mesenchymal stem cells in a chitosan/PCL/Zn scaffold for bone tissue regeneration. 2.The scaffold preparation involved the use of chitosan and PCL solution, freeze-drying, lyophilization, and treatment with Zinc solution. 3.FTIR analysis showed the functional groups present in the scaffold, such as OH, CH, CO, NH amino groups, and C-O-C indicating the presence of anhydrous glucose ring structure. 4.The surface morphology of the scaffold, including porosity and zinc deposition, was examined to understand its characteristics for promoting osteoblast differentiation	1.Novel 3D scaffold showed improved pore size, porosity, and water uptake. 2.Compression modulus increased to 78 MPa by adding nHA. 3. Layer for osteoregeneration improved cell proliferation rate compared to chondral regeneration	The chitosan/PCL/Zn scaffold demonstrated potential in promoting osteogenic differentiation of mesenchymal stem cells derived from ovarian follicular fluid, indicating its suitability for bone tissue engineering applications
8	Fatemeh Hejazi[29]/2021	IN VITRO	1.Novel 3D-functionality graded nanofibrous scaffolds were fabricated for osteochondral tissue regeneration using compositions like polycaprolactone, gelatin, nanohydroxyapatite, chitosan, and polyvinylalcohol. 2.The scaffolds showed improved physicochemical properties, increased water uptake capacity, and enhanced cell proliferation	1.Gravimetry method was used to measure total porosity of the scaffolds. 2.Fourier-transform infrared spectroscopy, X-ray diffraction, energy dispersive X-ray spectroscopy, scanning electron microscopy, mechanical compression test, porosimetry, and water uptake studies were applied to study the physicochemical properties	□ Novel 3D scaffold showed improved pore size, porosity, and water uptake. □ Compression modulus increased to 78 MPa by adding nHA. □ Layer for osteoregeneration improved	1.The novel 3D-functionality graded nanofibrous scaffolds PCL/gel & Cs/PVA showed promising potential for the treatment of osteochondral defects. The scaffolds exhibited improved physicochemical properties, increased water uptake capacity, and enhanced cell proliferation rate,

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
			rate, making them promising for treating osteochondral defects	of each layer and the whole scaffold. 3.FTIR and EDX analysis were used to investigate the chemical composition of the scaffold	cell proliferation rate compared to chondral regeneration	indicating their suitability for osteochondral tissue regeneration
9	Shizhou Wu[30]/ 2020	IN VITRO	1.The paper presents an injectable calcium phosphate scaffold with antibacterial properties against Staphylococcus aureus, supporting stem cells for bone regeneration. 2.The novel scaffold developed combines CPC-chitosan with alginate microbeads for sustained penicillin release, demonstrating good mechanical strength, antibacterial efficacy, and support for human umbilical cord mesenchymal stem cells viability	1.Antibiotic drug release profiles were analyzed, showing controlled release by diffusion and degradation. 2.The release profiles of antibiotics from alginate microbeads and CPC-chitosan-alginate microbeads were characterized by an initial burst release followed by a steady decrease in drug release. 3.Statistical analysis methods such as Bartlett's test, Shapiro-Wilk test, one-way ANOVA analysis, and Tukey's multiple comparison tests were used to analyze the data variance and significant differences in variables	1.Scaffold released 50% penicillin in 5 days, with long-term release. 2. Antibiotic release controlled by diffusion and degradation. Initial burst release of 80% loaded drug from alginate microbeads.	The injectable CPC-chitosan-penicillin-microbeads scaffold demonstrated potent antibacterial effects against S. aureus and supported stem cell growth for bone regeneration. The scaffold exhibited good mechanical strength comparable to cancellous bone and sustained release of penicillin for long-term antibacterial activity. The addition of penicillin did not compromise the injectability of the scaffold, showing promise for clinical applications in treating bone infections
10	Mohammad Porgham Daryasari[31]/ 2019	In Vitro	1.Composite scaffolds of PLLA incorporated Dexa-loaded MSN-CS were fabricated for enhanced osteogenic differentiation in bone tissue engineering applications. 2.The scaffolds showed improved osteogenic potential, conductive surface properties, and controlled drug release, making them ideal for pH-sensitive drug delivery in tissue regeneration	1.The study utilized Poly-l-lactic acid (PLLA) incorporated dexamethasone (Dexa) loaded Mesoporous Silica Nanoparticles (MSN) coated with Chitosan (CS) for fabricating composite scaffolds. 2.Tensile strength data was collected to evaluate the mechanical properties of the scaffolds, showing an increase in tensile stress and breaking tensile strain with the addition of MSN-DexaCS to PLLA scaffolds. 3.Statistical analysis was conducted using two-way analysis of variance (two-way ANOVA) with a	□ composite scaffolds enhanced osteogenic potential for bone tissue engineering. □ Controlled release of Dexa from scaffolds supported osteogenesis.	The composite scaffolds prepared in the study demonstrated enhanced osteogenic potential and controlled drug release, making them promising for bone tissue engineering applications. The incorporation of Dexa-loaded MSN-CS into PLLA scaffolds resulted in improved mechanical properties and osteogenic differentiation potential.

Sno	Author/year	Type of Study	Abstract	Parameters measured	values	conclusion
				significance level of p < 0.05		
11	Premjit Arpornmaeklong[32]/ 2021	IN VITRO	1.Thermosensitive chitosan/collagen hydrogels with quercetin showed optimal properties for bone tissue engineering and sustained release of natural flavonoids. 2.Incorporating quercetin in the chitosan/collagen hydrogel enhanced a bioactive microenvironment supporting stem cell encapsulation	1.The study utilized data on the physical, mechanical, and antioxidant properties of thermosensitive chitosan/collagen hydrogels, as well as the release profiles of quercetin from the hydrogels. 2.Mass spectrum spectrophotometry analysis results were used to determine the relative counts of mass fragments of quercetin in different concentrations of chitosan/collagen hydrogel. 3.Data on the pore diameters, pH levels, and structural properties of the chitosan/collagen hydrogels were analyzed to understand their characteristics and biologic effects. 4.Fourier transform infrared spectroscopy was employed to examine the incorporation of quercetin in the chitosan/collagen matrix	□ Quercetin-bGP-2:1 chitosan/collagen hydrogels promoted hPDLSC growth. □ Chitosan content improved porous structure and decreased quercetin release rate. □ Highest weight loss on Day 14 found in collagen group. □ Levels of cell viability in 2.5-10 mM quercetin were significantly higher.	The thermosensitive quercetin-bGP-2:1 chitosan/collagen hydrogel demonstrated optimal properties for bone tissue engineering and sustained release of natural flavonoids. The chitosan/collagen-quercetin hydrogels were expected to be osteoconductive, degradable, and bioactive, suitable for bone tissue engineering
12	Agata Przekora[33] / 2021	Ex vivo	1.The study presents ex vivo determination of osseointegration using human trabecular bone explants filled with chitosan/curdlan/hydroxyapatite biomaterial, demonstrating the formation of a connection with bone tissue. 2.The research highlights the use of human bone explants as a model to test biomaterial osseointegration, reducing the need for animal tests and aligning with the principles of '3Rs'	1.The study utilized human trabecular bone explants filled with chitosan/curdlan/hydroxyapatite biomaterial and cultured for at least 46 days. 2.Optical microscope imaging was used to observe the outgrowth of osteoblasts, leukocytes, and adipocytes during the culture period. 3.The research was supported by the Ministry of Science and Higher Education in Poland and various grants, emphasizing the significance of the findings	1.Osteoblasts detected at bone-implant interface, confirming osseointegration. 2. Biomaterial integration with bone explant observed through LiveDead staining. 3. Ex vivo bone explant model proved reliable for osseointegration determination.	Chitosan/curdlan/HA biomaterial found to exhibit osseointegration with human trabecular bone explants, with viable osteoblast, showing the formation of a connection with the biomaterial.

4. Discussion

This systematic review aimed to critically evaluate the current evidence on the osteogenic and osteo-inductive properties of chitosan-based composites in bone tissue regeneration. The analysis was grounded in a rigorous methodological approach, involving a comprehensive literature search across multiple databases and grey literature, which ensured the inclusion of a broad spectrum of relevant studies. In-vivo animal studies by far have been used to perform drug development for bony defect or diseases. However, these preclinical studies lead to poor translation of results to clinical trials. Hence, In-vitro studies have an upper hand in terms of controlling the testing environment for preclinical studies.[35,36] By incorporating both in vitro studies, the review offers a balanced perspective on the potential applications of chitosan in promoting bone healing.

In this systematic review the terms such as osteogenic potential, osteo-conduction and osteo-induction were used throughout the illustration and hence, its essential to understand their definition. Osteogenesis is the process of bone formation by cells that arise from primary cells, embryonic stem cells, MSCs (mesenchymal stem cells), and induced pluripotent stem cells. Osteo-conduction is the process by which bone grows on a surface or into a structure, such as an implant. Osteo-conduction is the capacity of a material to promote bone growth. Osteo-conductive materials do not have osteogenic properties, but they can serve as a scaffold for new bone to form. Osteo-induction is process of stimulating osteogenesis by recruiting osteogenic cells to the site of an injury. Osteo-induction is a key part of bone healing, and is responsible for most new bone formation.

The studies included in this review collectively suggest that chitosan, particularly when used in combination with other biomaterials such as hydroxyapatite[37,38] or bioactive glass[39], can significantly enhance the osteogenic differentiation of precursor cells and promote bone matrix deposition. The bone regeneration capacity of chitosan-based composites is particularly noteworthy, as evidenced by their ability to induce mesenchymal stem cell differentiation into osteoblasts and promote new bone formation in animal models. This aligns with existing literature that underscores the role of chitosan in creating a supportive environment for bone tissue engineering, facilitating cell adhesion, proliferation, and differentiation.

The methodological rigor of the included studies varied, with most exhibiting a moderate risk of bias. The use of the QUINN assessment tool allowed for a detailed evaluation of risk across several key domains, including randomization, blinding, and statistical analysis. The moderate risk of bias observed in most studies likely stems from the inherent challenges of in vitro research, such as difficulties in achieving randomization and blinding.[34] Additionally, some studies lacked detailed sample size calculations, potentially affecting the generalizability of their findings. The exclusion of studies with a high risk of bias was necessary to ensure the reliability of the review's conclusions, although it did limit the pool of available data. This highlights the need for more robustly designed studies in this field, with future research ideally focusing on randomized controlled trials in clinical settings to validate the promising in vitro results.

The findings of this review are consistent with existing literature on the role of chitosan in bone tissue regeneration.[37,39] Previous systematic reviews have reported similar outcomes, noting the potential of chitosan-based materials to promote osteogenesis and support bone healing. This review contributes to the body of evidence by specifically focusing on the composite nature of these materials, emphasizing the synergistic effects that can be achieved by combining chitosan with other bone regenerative agents.

One of the key insights from this review is the importance of composite structures in enhancing the bone regeneration in terms of osteoinduction, osteo-conduction and osteogenic potential of chitosan. The included studies consistently demonstrated that chitosan-based composites outperformed chitosan alone in promoting bone tissue regeneration. This observation is supported by mechanistic studies

showing that incorporating osteoconductive elements, such as calcium phosphate, into the chitosan matrix creates a more favourable microenvironment for bone formation.

The combination of chitosan with other materials was found to enhance its bioactivity, particularly in terms of osteo-inductivity and osteo-conductivity. For instance, Qiu et al. (2021) demonstrated that a chitosan-reinforced calcium phosphate scaffold not only promoted osteogenesis but also exhibited antimicrobial properties, making it a dual-functional scaffold for bone regeneration.[16]

Despite this, only a limited number of studies were ultimately included, reflecting the specificity of the inclusion criteria and the relatively niche focus of the research question. The mechanism proposed for the chelation of calcium ions by chitosan namely the bridge and the pendant model[37], states that two or more amino groups of chitosan bind to the same metal ion and in pendant model suggests that one amino group is utilized in the binding, and the metal ion is linked to the amino group like a pendant. Either of the two mechanisms could be responsible for the chelation of calcium ions. Other properties like antimicrobial properties, and fibroblast synthesis aid in better wound healing.

Three of the included studies described the cell adhesion and cellular proliferation indicating osteogenic potential of chitosan in combination either of genipin hydrogel[22], CaCo3 [23], ans Rosuvastin/ chondrointin sulfate scaffold.[24]

Studies by Gentao Qui[25](2021) demonstrated that chitosan with calcium phosphate and doxycycline had antibacterial effect on *Staphalococcus.Aureus* and *P.Gingivalis*. Chitosan is biocompatible and readily acceptable by tissues, which was validated by Piotr Kowalczyk et al [26](2021) in their study using chitosan calcium phosphate human bone composite showing non cytotoxic effect as well as high alkanine phosphatase activity, making them suitable for bone tissue engineering. For anti-inflamatory effect chitosan-based scaffold study by Paulino Kazimierczak [27](2021) demonstrated that chitosan / agarose / nanoHA bone scaffold induced M2 macrophage polarization, releasing anti-inflammatory cytokines and enhancing osteogenic differentiation.

Chitosan seemed to have better adaptability with flavonoids such as Quercitin, a flavonoid mostly found in onion which has anti-inflammatory and healing potential, was demonstrated by Premjit Arpormaeklong[32] et al (2021) in their study.

The clinical implications of these findings are significant. Chitosan-based composites hold promise as a viable alternative to traditional bone graft materials, particularly in situations where autografts or allografts are not feasible. The biocompatibility, biodegradability, and inherent bioactivity of chitosan make it an attractive candidate for bone tissue engineering applications. Furthermore, the ability to tailor the composite structure to enhance specific properties, such as osteo-inductivity or mechanical strength, increases the clinical utility of these materials. However, translating these promising in vitro results into clinical practice requires further investigation. Future studies should focus on large-scale clinical trials to establish the safety and efficacy of chitosan-based composites in human patients. Additionally, research should explore the long-term outcomes of using these materials in bone regeneration, including their integration with native bone tissue and the potential for inducing immune responses.

This underscores the osteogenic and osteo-inductive potential of chitosan-based composites in bone tissue regeneration. While the current evidence is promising, particularly in preclinical models, further research is necessary to fully realize the clinical applications of these materials. The moderate risk of bias identified in most studies highlights the need for more rigorously designed research, which will be essential for advancing the field of bone tissue engineering and improving patient outcomes.

5. Limitation(s)

Limitations of this study were that only in-vitro studies were selected for the review, their clinical relevance data are scarce or yet to be assessed. Since chitosan is a recent biomaterial in the field of

orthopaedics and dentistry, very few literatures are available for its clinical validation at bone level.

Some studies included in the review process had multiple parameters and materials, and in few studies sample sizes were not mentioned, and hence were not included in the risk bias. Validity of the studies and the interpretation of the results are reduced by the methodological weakness and this may lead to biased findings. In general, the quality of the included studies was quite low as only six among the 12 studies had moderate risk of bias while all others had a high risk of bias.

6. Conclusion

This systematic review highlights the promising osteoconductive and osteo-inductive properties of chitosan-based composites in bone tissue regeneration. The studies reviewed provide compelling evidence that chitosan, especially when combined with other osteoconductive and osteo-inductive materials, can significantly enhance bone formation by promoting the differentiation of precursor cells and supporting bone matrix deposition. While these findings are encouraging, they are predominantly based on in vitro studies and preclinical models, which, although informative, require further validation through well-designed clinical trials.

Despite the moderate risk of bias present in many of the included studies, the potential of chitosan-based composites as an alternative to traditional bone graft materials remains evident. The ability to customize these composites to improve specific properties, such as mechanical strength and osteo-inductivity, positions them as a versatile option in bone tissue engineering. However, for these materials to be successfully integrated into clinical practice, additional research is needed to address the existing methodological limitations and confirm their long-term efficacy and safety in human applications.

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