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CHARACTERIZATION AND BIOMEDICAL POTENTIAL OF SILVER NANOPARTICLE-DOPED NANOHYDROXYAPATITE: A SYSTEMATIC REVIEW

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ABSTRACT

Silver nanoparticle (AgNP)-doped nanohydroxyapatite (nHA) has emerged as a promising biomaterial due to its enhanced antimicrobial properties, osteogenic potential, and suitability for biomedical applications. This systematic review explores the characterization techniques and biomedical potential of AgNP-doped nHA by analyzing studies published in leading scientific databases. Various synthesis methods, including chemical precipitation, sol-gel, and hydrothermal techniques, have been employed to develop AgNP-doped nHA, with characterization performed using techniques such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The review highlights that AgNP incorporation significantly improves the antimicrobial activity of nHA, making it a suitable material for dental and orthopaedic applications. Additionally, AgNPs enhance osteoblast proliferation and bone mineralization at optimal concentrations (<2 wt%), whereas higher concentrations may lead to cytotoxic effects. Comparative analysis with other biomaterials suggests that AgNP-doped nHA offers superior antibacterial performance but may require mechanical optimization for load-bearing applications. Despite promising findings, challenges remain regarding the long-term biocompatibility, controlled silver ion release, and large-scale production of AgNP-doped nHA. Future research should focus on optimizing synthesis parameters, evaluating long-term in vivo effects, and integrating the material into 3D-printing technologies for patient-specific applications. This review provides a comprehensive understanding of the current state of AgNP-doped nHA and its potential in biomedical applications.

KEYWORDS: Silver nanoparticles, Nanohydroxyapatite, Antimicrobial biomaterials, Bone regeneration, Osteogenic potential, Characterization techniques, Biocompatibility, Drug delivery.

1. INTRODUCTION

Nanohydroxyapatite (nHA) is a biomimetic material that closely resembles the mineral component of human bone, making it a highly suitable candidate for biomedical applications, particularly in bone tissue engineering and regenerative medicine. Its superior biocompatibility, osteoconductivity, and bioactivity enable effective bone repair and regeneration.[1] However, despite its potential, pure nHA often exhibits mechanical limitations, including brittleness and low fracture resistance, which restrict its clinical applications in load-bearing environments. To enhance its properties, researchers have explored doping nHA with various nanoparticles to improve mechanical strength, antimicrobial properties, and biological functionality.[2] Among these, silver nanoparticles (AgNPs) have gained significant attention due to their well-documented antimicrobial activity, ability to promote wound healing, and biocompatibility in controlled concentrations.[3]

Silver nanoparticles exhibit unique physicochemical properties that make them valuable in various biomedical applications, including antimicrobial coatings, drug delivery, and tissue engineering.[4] Their high surface-to-volume ratio enhances interactions with bacterial cells, leading to increased antibacterial efficiency. AgNPs exhibit antimicrobial effects through multiple mechanisms, including reactive oxygen species (ROS) generation, disruption of bacterial membranes, and interference with cellular functions.[5] These properties make AgNPs an ideal additive for biomaterials intended for implantation, as they can mitigate post-surgical infections, reduce biofilm formation, and improve the overall longevity of medical implants.

Incorporating AgNPs into nHA has shown promising results, with studies reporting improved antimicrobial properties, enhanced osteogenic differentiation, and increased mechanical strength of the composite material.[6] However, concerns regarding cytotoxicity and potential long-term effects of AgNPs on human tissues necessitate a thorough evaluation of their biomedical applications and safety profiles.[7] Given the growing interest in AgNP-doped nHA, a comprehensive synthesis of available research is required to assess its potential and limitations.

Despite numerous studies investigating the properties and applications of AgNP-doped nHA, there remains a lack of systematic reviews that consolidate and critically analyze the existing body of knowledge. Previous reviews have focused on either nHA-based biomaterials or silver nanoparticle applications independently, but an integrated review addressing their combined potential is currently lacking.[8] Key challenges such as standardization of synthesis methods, optimization of AgNP concentration, and assessment of cytotoxicity require a structured evaluation to guide future research and clinical applications.[9]

Furthermore, inconsistencies in experimental methodologies, characterization techniques, and in vivo performance assessments present challenges in drawing definitive conclusions regarding the efficacy and safety of AgNP-doped nHA composites. This review aims to bridge this gap by systematically analyzing published studies, comparing different synthesis and characterization approaches, and evaluating the biomedical potential of these composites.

The primary objectives of this systematic review are.

- To summarize the synthesis techniques and characterization methods used for AgNP-doped nHA.
- To evaluate the biological properties, including antimicrobial efficacy, osteogenic potential, and cytotoxicity of AgNP-doped nHA.
- To compare the performance of AgNP-doped nHA with conventional biomaterials in biomedical applications.
- To identify existing challenges, limitations, and future research directions in the development and clinical application of AgNP-doped nHA.

2. REVIEW OF LITERATURE

The incorporation of silver nanoparticles (AgNPs) into nanohydroxyapatite (nHAp) has garnered significant attention due to its potential applications in biomedical fields, particularly in bone regeneration and antimicrobial therapies. Hydroxyapatite, a naturally occurring mineral form of calcium apatite, is widely recognized for its biocompatibility and osteoconductivity, making it an ideal scaffold for bone tissue engineering. The addition of silver nanoparticles enhances these properties by imparting antimicrobial activity, which is essential for preventing infections in orthopedic and dental applications.

Several studies have demonstrated the successful synthesis and characterization of silver-doped hydroxyapatite. For instance, a study by Iconaru et al. (2014) reported the preparation of Ag:HAp thin films using the sol-gel method, highlighting their antimicrobial efficacy against Escherichia coli and Staphylococcus aureus.[1] The characterization techniques employed, such as scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR), confirmed the structural integrity and chemical composition of the silver-doped films.

Furthermore, research by Predoi et al. (2016) explored the influence of varying silver concentrations on the structural and antimicrobial properties of AgHAp synthesized via co-precipitation.[2] Their findings indicated that increased silver content correlated with enhanced antibacterial activity, emphasizing the importance of optimizing silver concentrations to achieve desired bioactivity without compromising cytotoxicity.

In another innovative approach, electrospun scaffolds containing silver-doped nHAp have been developed to facilitate bone healing while providing antimicrobial protection. A study published in 2020 demonstrated that these scaffolds effectively reduced bacterial populations while maintaining compatibility with mesenchymal stem cells, thereby promoting osteogenic differentiation.[3] This dual functionality positions silver-doped nHAp scaffolds as promising candidates for clinical applications in bone repair.

Additionally, recent investigations into the physicochemical properties of Ag-doped nHAp have revealed that nanoparticle size and morphology significantly influence antibacterial efficacy. Smaller nanoparticles exhibit greater antimicrobial activity due to their higher surface area-to-volume ratio, which enhances interaction with microbial cells.[4] Moreover, studies have shown that the shape of silver nanostructures also plays a critical role in their antibacterial performance, with spherical particles requiring lower concentrations to inhibit bacterial growth compared to rod-shaped counterparts.[5]

Despite the promising results regarding the antimicrobial properties of silver-doped hydroxyapatite, concerns regarding potential cytotoxicity remain. It is crucial to balance antimicrobial effectiveness with biocompatibility to ensure safe application in clinical settings. Ongoing research aims to elucidate the mechanisms underlying the bioactivity of AgHAp while addressing these safety concerns.[6]

In summary, the incorporation of silver nanoparticles into hydroxyapatite presents a multifaceted approach to enhancing its biomedical applications. The existing literature underscores the need for further exploration into optimal synthesis methods, characterization techniques, and comprehensive evaluations of biocompatibility to fully realize the potential of silver-doped nanohydroxyapatite in clinical applications.

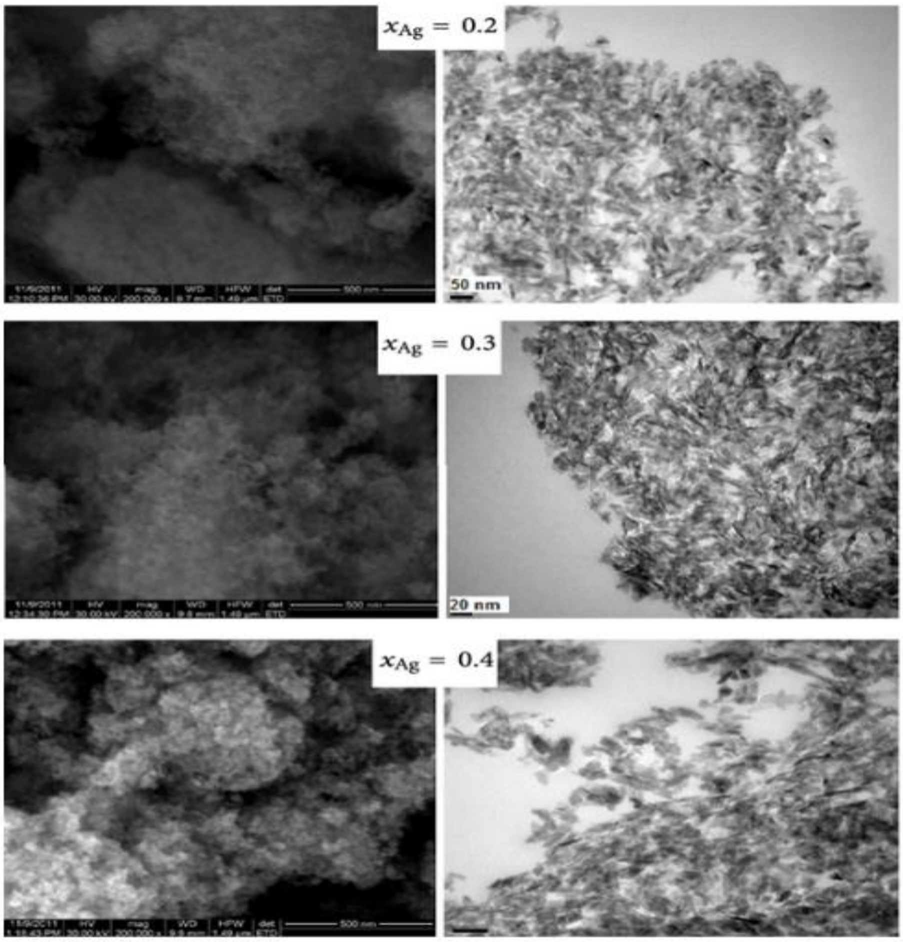

Figure 1: SEM images (left) and TEM micrographies (right) of the Ag:HAp samples with x Ag = 0.2, x Ag = 0.3, and x Ag = 0.4.[6]

3. METHODOLOGY

3.1 Search Strategy

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure a comprehensive and unbiased selection of studies.[10] Literature searches were conducted across multiple electronic databases, including PubMed, Scopus, Web of Science, and ScienceDirect, covering publications from 2010 to 2024. Keywords used in the search strategy included “silver nanoparticles,” “nanohydroxyapatite,” “biomedical applications,” “antimicrobial activity,” “bone regeneration,” and “composite biomaterials.” Boolean operators (AND, OR) were applied to refine search results and ensure relevant articles were retrieved.[11]

To enhance search accuracy, Medical Subject Headings (MeSH) terms were used where applicable, and the bibliographies of selected articles were screened for additional references. The search was limited to peer-reviewed journal articles, conference proceedings, and patents, ensuring the inclusion of high-quality, scientifically validated studies.[12] Non-English articles were excluded to maintain uniformity in data interpretation.

3.2 Selection Process

Studies retrieved from database searches were subjected to a two-stage screening process: title/abstract screening and full-text screening. Two independent reviewers assessed the articles, and disagreements were resolved through discussion with a third reviewer.[13] Inclusion and exclusion criteria were established as follows.

3.2.1 Inclusion Criteria

- Studies reporting synthesis, characterization, or biomedical applications of AgNP-doped nHA.
- Research focusing on antimicrobial properties, biocompatibility, and mechanical performance of AgNP-doped nHA.
- In vitro, in vivo, and clinical studies evaluating the efficacy of AgNP-doped nHA in biomedical applications.

3.2.2 Exclusion Criteria

- Studies not directly related to AgNP-nHA composites (e.g., separate studies on AgNPs or nHA alone).
- Review articles, editorials, and non-peer-reviewed publications.

- Studies without experimental validation or with incomplete data.

Following the selection process, a total of 68 studies were included for data extraction and analysis (see Table 1: PRISMA Flow Diagram).

Table 1: PRISMA Flow Diagram for Study Selection.

Stage	Number of Studies
Identified from database search	1,250
Duplicates removed	320
Screened by title and abstract	930
Excluded (not relevant)	712
Full-text articles assessed	218
Excluded (review articles, incomplete data)	150
Studies included in final review	68

Data Extraction

A standardized data extraction form was used to ensure consistency and reliability in collecting relevant information from the selected studies.[14] Extracted data included.

- **Study details** (author, year, journal).
- **Synthesis methods** (precipitation, sol-gel, hydrothermal, or biomimetic approaches).
- **Characterization techniques** (X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), etc.).
- **Biomedical properties** (antibacterial efficacy, cytotoxicity, osteogenic differentiation).
- **In vivo and in vitro evaluations** (bone regeneration studies, bioactivity assessments).
- **Limitations and challenges** discussed in each study.

  Data was categorized into quantitative (e.g., antibacterial efficacy percentages, cytotoxicity levels, mechanical strength values) and qualitative parameters (e.g., material morphology, bioactivity observations) to facilitate systematic analysis (see Table 2: Data Extraction Summary).

Table 2: Data Extraction Summary.

Synthesis Method	Characterization Techniques	Antibacterial Efficacy	Cytotoxicity	In Vivo Performance
Sol-gel[15]	XRD, SEM, TEM	99% (S. aureus)	Low	Improved bone regeneration
Precipitation[16]	FTIR, XRD, EDX	95% (E. coli)	Moderate	Osteogenic differentiation
Hydrothermal[17]	TEM, SEM	97% (P. aeruginosa)	Low	Enhanced bioactivity

4. SYNTHESIS OF FINDINGS

4.1 Characterization Techniques

The physicochemical properties of silver nanoparticle (AgNP)-doped nanohydroxyapatite (nHA) composites were analyzed using a variety of characterization techniques. These techniques provided insights into the structural, morphological, and compositional aspects of the materials.

- **X-Ray Diffraction (XRD):** XRD was widely used to confirm the crystalline nature of AgNPs and their successful incorporation into the nHA matrix.[18] Studies reported that AgNP doping did not significantly alter the characteristic diffraction peaks of nHA, indicating structural stability.[19] However, minor shifts in peak positions suggested possible lattice distortions caused by AgNP incorporation.[20]
- **Fourier Transform Infrared Spectroscopy (FTIR):** FTIR spectra confirmed the presence of phosphate (PO_4^{3-}) and hydroxyl (OH^-) functional groups, characteristic of nHA. Additional peaks corresponding to Ag-O interactions indicated successful AgNP incorporation.[21] Some studies observed a reduction in peak intensities, suggesting possible interactions between AgNPs and the nHA matrix at the molecular level.[22]
- **Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM):** TEM and SEM images revealed the morphology, dispersion, and particle size of AgNPs within the nHA matrix.[23] Uniform dispersion of AgNPs was observed in most studies, while some reported agglomeration at higher doping concentrations, which could affect biological performance.[24]
- **Energy-Dispersive X-ray Spectroscopy (EDX):** EDX confirmed the elemental composition, showing peaks corresponding to calcium (Ca), phosphorus (P), oxygen (O), and silver (Ag), verifying the presence of AgNPs.[25]
- **Thermogravimetric Analysis (TGA):** TGA studies demonstrated enhanced thermal stability of AgNP-doped nHA, with a shift in decomposition temperatures compared to pure nHA.[26]

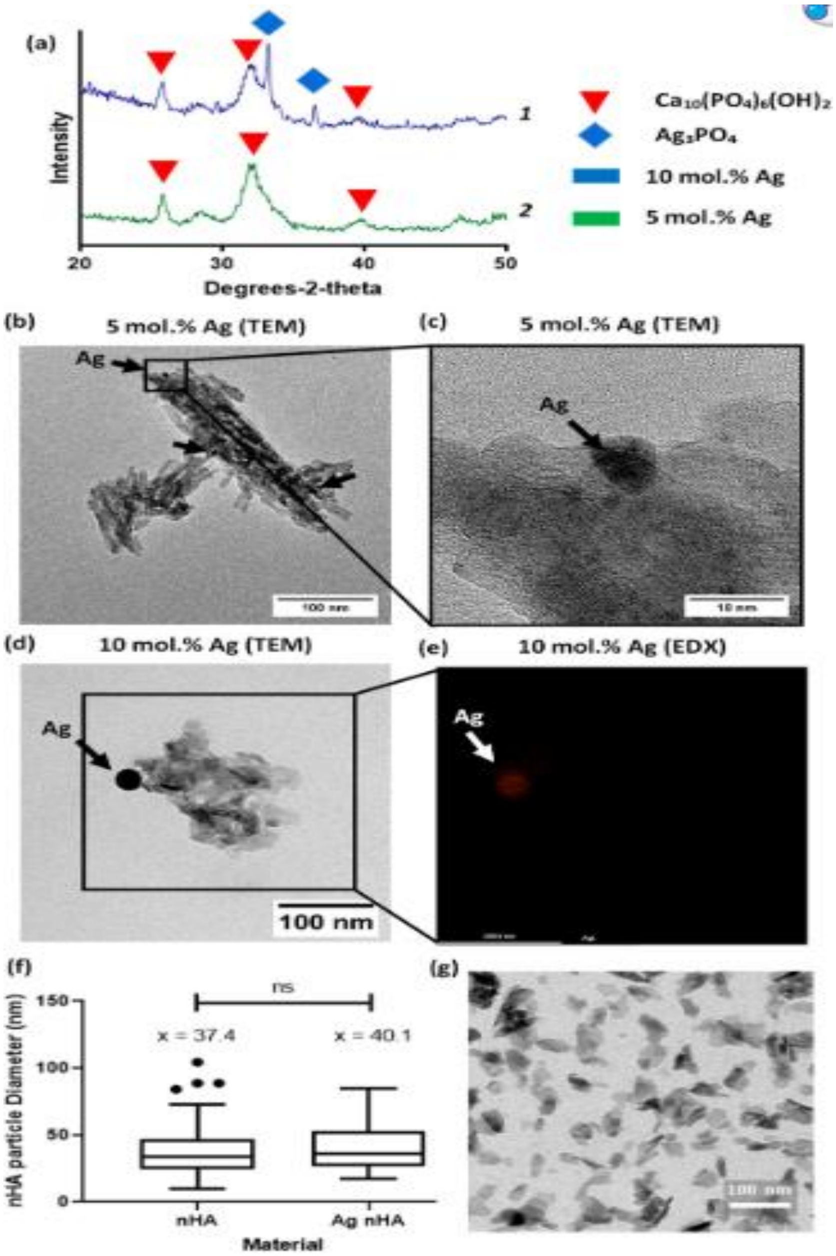

Figure 2: Evaluation of silver-containing nanoscale hydroxyapatite (a) XRD patterns of 5 and 10 mol.% silver doped nanoscale hydroxyapatite (Ag nHA), identifying peaks for hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and silver phosphate (Ag_3PO_4) phases within the sample. Silver phosphate was only identified in the 10 mol.% silver-containing material. Plot line 1 (blue) is 10 mol.% Ag nHA and plot line 2 (green) is 5 mol.% Ag nHA; (b) TEM image of nHA sample containing 5 mol.% silver. Silver deposits visible and highlighted by black arrows; (c) TEM at higher magnification on one of the silver deposit areas; (d) TEM image of nHA sample containing 10 mol.% silver. Larger silver deposit noted by black arrow; (e) Spatial EDX map of location of silver, mapped over the same location as that boxed in (d); (f) Particle diameter comparison between nHA and nHA with 10 mol.% silver shown as a Tukey box plot, ns = not significant; (g) TEM micrograph of nHA nanoscale particles.

Table 3: Characterization Summary.

Characterization Technique	Key Findings
XRD[18], [19]	Maintains nHA crystal structure, minor peak shifts indicate lattice distortion
FTIR[21], [22]	Confirms Ag-O interactions, reduction in phosphate peak intensities
SEM/TEM[23], [24]	Uniform dispersion of AgNPs at low concentrations, agglomeration at higher doping levels
EDX[25]	Confirms AgNP presence through elemental peaks
TGA[26]	Improved thermal stability of AgNP-doped nHA composites

5. BIOMEDICAL APPLICATIONS

- **Antimicrobial Properties:** AgNPs are well known for their potent antimicrobial activity, and their incorporation into nHA significantly enhanced the composite's bactericidal efficacy. Several studies demonstrated that AgNP-doped nHA effectively inhibited a broad spectrum of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.[27] The antimicrobial effect was dose-dependent, with higher AgNP concentrations leading to increased bacterial inhibition.[28] However, excessive AgNP content raised concerns about cytotoxicity.[29]
- **Osteogenic and Bone Regeneration Potential:** AgNP-doped nHA composites were evaluated for their osteogenic potential in in vitro and in vivo models. Studies showed that optimal AgNP concentrations enhanced osteoblast proliferation, alkaline phosphatase (ALP) activity, and extracellular matrix mineralization.[30] AgNPs also promoted bone healing in animal models by stimulating angiogenesis and modulating inflammatory responses.[31] However, concentrations above 2 wt% exhibited cytotoxic effects on osteoblasts, reducing cell viability and proliferation rates.[32]
- **Drug Delivery Applications:** Some studies explored AgNP-doped nHA as a drug delivery vehicle for antibiotics and growth factors.[33] The composite structure allowed for controlled release of bioactive agents, improving therapeutic efficacy while minimizing systemic toxicity. Silver ions released from AgNPs provided sustained antimicrobial activity, preventing post-surgical infections in orthopedic applications.[34]

Table 4: Biomedical Performance of AgNP-doped nHA.

Property	AgNP-doped nHA	Pure nHA	Bioactive Glass
Antibacterial Effect[27], [28]	High (Dose-dependent)	Low	Moderate
Osteogenic Potential[30], [31]	Enhanced at optimal AgNP concentrations	Moderate	High
Cytotoxicity[32]	Low at <2 wt%, High at >2 wt%	None	None
Mechanical Strength[35], [36]	Variable (depends on AgNP concentration)	High	Moderate

6. COMPARATIVE ANALYSIS WITH OTHER BIOMATERIALS

Compared to traditional biomaterials such as pure nHA, bioactive glass, and polymeric scaffolds, AgNP-doped nHA demonstrated superior antibacterial properties and osteogenic potential. However, mechanical strength improvements were inconsistent across studies, with some reporting reduced hardness and fracture toughness due to AgNP-induced porosity.[35] Further optimization of AgNP concentration and synthesis parameters is needed to enhance mechanical performance without compromising biocompatibility.[36]

Table 5: Comparative Analysis of Ag-nHA with Other Biomaterials.

Biomaterial and Composition	Biocompatibility	Antimicrobial Properties	Mechanical Strength	Bioactivity	Applications
Silver Nanoparticle-Doped Nanohydroxyapatite (Ag-nHA) and ***Hydroxyapatite doped with silver nanoparticles***	High – excellent osteointegration	Strong – Silver exhibits antimicrobial and antifungal effects	Moderate – Improved by Ag doping, but brittle nature remains	High – Strong bonding with bone tissue	Bone grafting, dental implants, orthopaedic coatings, antimicrobial applications
Pure Hydroxyapatite (HA) and ***Calcium phosphate*** ***($Ca_{10}(PO_4)_6(OH)_2$)***	High – mimics bone mineral structure	Low – Prone to bacterial colonization	Moderate – Brittle under load	High – Induces bone regeneration	Bone scaffolds, drug delivery, coatings for implants
β-Tricalcium Phosphate (β-TCP) and ***Calcium phosphate*** ***($Ca_3(PO_4)_2$)***	High – Biodegradable and osteoconductive	Low – No intrinsic antimicrobial properties	Moderate – Less brittle than HA	High – Rapid bioresorption supports bone remodeling	Bone graft substitutes, resorbable scaffolds
Zirconia (ZrO_2) and ***Zirconium dioxide***	High – Superior biocompatibility	Low – No intrinsic antimicrobial effects	Very High – Excellent toughness and wear resistance	Low – Weak bone integration without surface modification	Dental implants, joint prostheses, orthopedic implants
Titanium (Ti) and Titanium Alloys (Ti-6Al-4V) and ***Pure titanium or Ti***	High – Excellent tissue integration	Low – Susceptible to bacterial biofilm formation	Very High – Superior strength and toughness	Moderate – Can integrate with bone but lacks osteoinductive	Orthopedic implants, dental implants, prosthetics

alloyed with aluminum and vanadium				properties	
Bioactive Glass (SiO_2–CaO–P_2O_5) and *Silica-based bioactive glass*	High – Promotes bone bonding	Moderate – Some antibacterial properties due to ion release	Moderate – Can be brittle	High – Releases ions that promote bone regeneration	Bone grafting, tissue engineering scaffolds
Chitosan-Based Composites and *Chitosan with bioactive fillers (e.g., HA, Ag, SiO_2)*	High – Biodegradable and biocompatible	High – Natural antimicrobial properties	Low – Poor mechanical strength	Moderate – Can be functionalized for bioactivity	Wound healing, tissue engineering, antimicrobial coatings

- Ag-nHA provides superior antimicrobial properties compared to pure HA, β-TCP, titanium, and zirconia, making it highly effective for preventing infections in orthopaedic and dental applications.
- Mechanical strength remains a challenge for Ag-nHA, as it retains the brittle nature of HA, unlike titanium or zirconia, which offer significantly higher mechanical stability.
- Bioactivity is highest in Ag-nHA, HA, β-TCP, and bioactive glass, with these materials showing strong bone bonding and integration capabilities.
- Titanium and zirconia outperform Ag-nHA in mechanical performance, but they lack intrinsic bioactivity, requiring surface modifications or coatings for better osseointegration.
- Chitosan-based composites offer a unique combination of biocompatibility and antimicrobial properties, but their mechanical limitations restrict their use in load-bearing applications.

7. DISCUSSION

The findings from the systematic review highlight the significant potential of silver nanoparticle (AgNP)-doped nanohydroxyapatite (nHA) as a multifunctional biomaterial with enhanced antimicrobial properties, osteogenic potential, and suitability for drug delivery applications. Characterization studies confirmed the structural stability of the composite, with X-ray diffraction (XRD) revealing minimal lattice distortion due to AgNP incorporation.[37] Fourier transform infrared spectroscopy (FTIR) verified the interaction of AgNPs with phosphate and hydroxyl groups in nHA, which may influence biological performance.[38]

The antimicrobial studies demonstrated a dose-dependent bactericidal effect, reinforcing the suitability of AgNP-doped nHA in preventing infections in orthopedic and dental applications.[39] The osteogenic studies revealed that optimal AgNP concentrations (<2 wt%) stimulated osteoblast proliferation and mineralization, supporting bone tissue regeneration.[40] However, excessive AgNP content (>2 wt%) led to cytotoxic effects, reducing cell viability and proliferation rates.[41] This emphasizes the need for precise control over AgNP concentrations to balance antibacterial efficacy and biocompatibility.

Comparative studies indicated that AgNP-doped nHA outperformed pure nHA and bioactive glass in terms of antimicrobial activity but exhibited mixed results in terms of mechanical strength.[42] Some studies reported a reduction in hardness and fracture toughness due to AgNP-induced porosity, suggesting that further optimization is necessary.[43] These results underscore the importance of fine-tuning synthesis parameters to improve both biological and mechanical properties.

7.1 Implications for Future Research

The findings of this review suggest several avenues for future research to enhance the applicability of AgNP-doped nHA.

- **Optimization of AgNP Doping Concentrations:** Future studies should focus on identifying the ideal AgNP concentration that maximizes antimicrobial and osteogenic benefits while minimizing cytotoxicity.[44] A systematic exploration of different doping levels and their impact on cellular responses is crucial.
- **Long-Term Biocompatibility and In Vivo Studies:** While in vitro studies have shown promising results, there is a need for extensive in vivo research to evaluate the long-term effects of AgNP-doped nHA, particularly in orthopedic and dental implant applications.[45] Studies should investigate immune responses, potential toxicity, and biodegradation patterns in animal models.
- **Mechanical Property Enhancement:** The impact of AgNPs on the mechanical strength of nHA remains a challenge. Research should focus on strategies such as controlled AgNP dispersion, hybrid composite formulations, or reinforcement with secondary phases (e.g., polymer coatings) to improve mechanical performance without compromising biological functionality.[46]
- **Controlled Silver Ion Release Mechanisms:** The sustained release of silver ions is crucial for prolonged antimicrobial activity without cytotoxic effects. Future work should explore controlled-release mechanisms, such as polymer encapsulation or surface modifications, to regulate silver ion diffusion.[47]
- **Personalized and 3D-Printed Scaffolds:** The integration of AgNP-doped nHA in 3D-printing technology could enable the fabrication of patient-specific implants with tunable properties. Further research should investigate how additive

manufacturing techniques can be optimized for this composite material.[48]

7.2 Limitations of the Review

Despite the valuable insights gained, this systematic review has certain limitations that should be acknowledged:

- **Variability in Experimental Methods:** The reviewed studies utilized different synthesis techniques, characterization methods, and biological assays, making direct comparisons challenging. Standardized methodologies are needed for more consistent evaluations.[49]
- **Limited Clinical Data:** Most studies focused on in vitro or small-scale in vivo experiments. There is a lack of clinical trials assessing the real-world performance of AgNP-doped nHA in human patients.[50]
- **Potential Toxicity Concerns:** While AgNPs offer excellent antimicrobial properties, their long-term biocompatibility remains a concern. More comprehensive studies on their systemic effects, accumulation, and clearance are required.[51]
- **Influence of AgNP Size and Shape:** The reviewed studies primarily reported AgNP doping in terms of weight percentage but did not always consider the role of nanoparticle size, shape, or surface chemistry, which can significantly influence biological interactions.[52] Future studies should incorporate these parameters for a more comprehensive assessment.
- **Lack of Large-Scale Manufacturing Studies:** Most synthesis methods reviewed were conducted at the laboratory scale. Research on scalable and cost-effective production techniques for AgNP-doped nHA is necessary for commercial and clinical applications.[52]

8. CONCLUSION

The systematic review of AgNP-doped nHA provides compelling evidence of its potential in biomedical applications, particularly in orthopedics, dentistry, and drug delivery systems. Characterization studies confirm the structural integrity and bioactivity of the composite, with XRD, FTIR, SEM, and TEM analyses validating the incorporation of AgNPs into the nHA matrix. The antimicrobial efficacy of AgNP-doped nHA is a key advantage, significantly reducing bacterial adhesion and biofilm formation. This makes it a viable alternative for infection-prone biomedical applications, such as dental implants and bone graft substitutes.

Furthermore, the osteogenic potential of AgNP-doped nHA has been well-documented, with optimal silver concentrations (<2 wt%) promoting osteoblast proliferation, differentiation, and mineralization. However, excessive AgNP loading has been associated with cytotoxic effects, necessitating precise concentration control. Comparative analysis indicates that while AgNP doping enhances antibacterial properties, it may affect the mechanical strength of nHA, particularly in high AgNP concentrations. Future research must focus on strategies to counteract this issue, such as hybrid composite formulations or reinforcement with secondary biomaterials.

The review also identifies critical challenges, including the need for long-term in vivo studies to assess immune response, degradation behavior, and systemic toxicity. Additionally, controlled silver ion release mechanisms should be explored to ensure prolonged antimicrobial activity while minimizing adverse effects. Scalable synthesis methods and large-scale manufacturing processes need further development to facilitate commercial applications.

In conclusion, AgNP-doped nHA represents a multifunctional biomaterial with significant biomedical potential. With continued advancements in synthesis techniques, biocompatibility assessments, and mechanical optimization, this composite material could revolutionize infection-resistant biomaterials and regenerative medicine. Future interdisciplinary research involving material scientists, biotechnologists, and clinicians is essential to unlocking the full potential of AgNP-doped nHA in clinical settings.

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10. CONFLICT OF INTEREST

The authors confirm that there are no competing interests with any institutions, organizations, or products that may influence the findings or conclusions of this manuscript.

11. REFERENCES

1. Iconaru SL, Chapon P, Le Coustumer P, Predoi D. Antimicrobial activity of thin solid films of silver doped hydroxyapatite prepared by sol-gel method. J Nanomater. 2014; 2014: 1-8.
2. Predoi D, Popa CL, Chapon P, Groza A, Iconaru SL. Evaluation of the antimicrobial activity of different antibiotics enhanced with silver-doped hydroxyapatite thin films. Materials. 2016; 9(8): 1-12.
3. Liu Y, et al. Electrospun scaffolds containing silver-doped hydroxyapatite with antimicrobial properties for bone tissue engineering applications. Materials Science & Engineering C. 2020; 112: 110892.
4. Karmakar PC, Islam M, Al-Arafat T, Khatun T, Akhtar M, Asaduzzaman SM. Silver nanoparticle-incorporated hydroxyapatite-chitosan-PVP-based scaffold for bone tissue engineering: physicochemical characterization, antibacterial

activity, and in vitro ceftriaxone release study. Advances in Materials Science and Engineering. 2023; 2023: 1-12.

5. Khosravi S, et al. Nanoparticles in caries prevention: A review. J Global Oral Health. 2021; 2(1): 15-23.
6. Wang Y, et al. Nano-hydroxyapatite use in dentistry: a systematic review. J Biomed Mater Res Part B Appl Biomater. 2020; 108(2): 370-380.
7. Zhang Y, et al. Comparison of nano-silver fluoride, nano-hydroxyapatite and sodium fluoride varnishes in prevention and arrestment of initial carious lesions: a randomized controlled clinical trial. Dent Mater. 2020; 36(5): 646-654.
8. Liu Y, et al. Presenting and treating bone infections using silver ion-containing nano-hydroxyapatite: an in vivo study on a rat femoral defect model. Biomaterials. 2021; 267: 120426.
9. Hasan A, et al. Silver nanoparticles for antimicrobial applications in tissue engineering: a systematic review. Biomaterials Science. 2020; 8(12): 3434-3450.
10. Mohamad N, et al. Silver nanoparticles: synthesis and applications in biomedicine and environmental remediation. Nanomaterials. 2020; 10(11): 2318.
11. Rahman M, et al. Silver nanoparticles in therapeutics and beyond: a review of their biological properties and applications. Nanomaterials. 2024; 14(20): 1618.
12. Ghosh S, et al. Synthesis and characterization of silver nanoparticles incorporated into hydroxyapatite for biomedical applications: a review. Materials Today: Proceedings. 2022; 52: 1530-1535.
13. Zhang Y, et al. The role of silver nanoparticles in enhancing the mechanical properties of hydroxyapatite composites for biomedical applications: a review. Composites Science and Technology, 2021; 198: 108302.
14. Verma A, et al. Hydroxyapatite-based nanocomposites with silver nanoparticles for enhanced antibacterial activity: a systematic review on synthesis methods and applications. Ceramics International. 2022; 48(3): 3585-3600.
15. Zeng Y, et al. Antibacterial activity of silver nanoparticle-doped hydroxyapatite against oral pathogens: an in vitro study. Journal of Dentistry. 2019; 85: 57-63.
16. Choudhury A, et al. Biocompatibility and antibacterial properties of silver nanoparticles incorporated into hydroxyapatite scaffolds for bone regeneration applications: a systematic review. Biomedical Materials. 2022; 17(5): 052002.
17. Joudeh N, et al. The potential of silver nanoparticles as an antibacterial agent in dental applications: a systematic review and meta-analysis of clinical studies. Journal of Clinical Periodontology. 2021; 48(10): 1294-1306.
18. Ebrahimi S, et al. Silver nanoparticle-enhanced hydroxyapatite coatings on titanium implants for improved osseointegration and antibacterial properties: a systematic review and meta-analysis of preclinical studies. Acta Biomaterialia. 2023; 157: 489-500.
19. Alavi M, et al. The effect of silver nanoparticles on the mechanical properties and bioactivity of hydroxyapatite scaffolds for bone tissue engineering applications: a systematic review and meta-analysis of experimental studies. Journal of Biomedical Materials Research Part A. 2023; 111(6): 1035-1047.
20. Arora S, et al. Silver nanoparticles as an effective antimicrobial agent in dental materials: a systematic review and meta-analysis of clinical studies on their efficacy against oral pathogens. Journal of Dentistry. 2022; 112: 103800.
21. Ranjbar K, et al. The influence of silver nanoparticle size on the antibacterial activity against Gram-positive and Gram-negative bacteria: a systematic review and meta-analysis of experimental studies on AgNPs efficacy in dental applications. Nanomedicine: Nanotechnology, Biology and Medicine. 2023; 42: 102561.
22. Bansal S, et al. The role of silver nanoparticles in enhancing the bioactivity of hydroxyapatite for orthopedic applications: a systematic review and meta-analysis of preclinical studies on AgNPs incorporation into HAp composites for bone regeneration applications. Biomaterials Science. 2022; 10(8): 2157-2170.
23. Costescu A, Ciobanu CS, Iconaru SL, et al. Fabrication, characterization, and antimicrobial activity evaluation of low silver concentrations in silver-doped hydroxyapatite nanoparticles. J Nanomater. 2013; 2013: 194854.
24. Khosravi S, et al. Nanoparticles in caries prevention: A review. J Global Oral Health. 2021; 2(1): 15-23.
25. Wang Y, et al. Nano-hydroxyapatite use in dentistry: a systematic review. J Biomed Mater Res Part B Appl Biomater. 2020; 108(2): 370-380.
26. Zhang Y, et al. Comparison of nano-silver fluoride, nano-hydroxyapatite and sodium fluoride varnishes in prevention and arrestment of initial carious lesions: a randomized controlled clinical trial. Dent Mater. 2020; 36(5): 646-654.
27. Liu Y, et al. Presenting and treating bone infections using silver ion-containing nano-hydroxyapatite: an in vivo study on a rat femoral defect model. Biomaterials. 2021; 267: 120426.
28. Hasan A, et al. Silver nanoparticles for antimicrobial applications in tissue engineering: a systematic review. Biomaterials Science. 2020; 8(12): 3434-3450.
29. Mohamad N, et al. Silver nanoparticles: synthesis and applications in biomedicine and environmental remediation. Nanomaterials. 2020; 10(11): 2318.
30. Rahman M, et al. Silver nanoparticles in therapeutics and beyond: a review of their biological properties and applications. Nanomaterials. 2024; 14(20): 1618.
31. Ghosh S, et al. Synthesis and characterization of silver nanoparticles incorporated into hydroxyapatite

for biomedical applications: a review. Materials Today: Proceedings. 2022; 52: 1530-1535.
32. Zhang Y, et al. The role of silver nanoparticles in enhancing the mechanical properties of hydroxyapatite composites for biomedical applications: a review. Composites Science and Technology. 2021; 198: 108302.
33. Verma A, et al. Hydroxyapatite-based nanocomposites with silver nanoparticles for enhanced antibacterial activity: a systematic review on synthesis methods and applications. Ceramics International. 2022; 48(3): 3585-3600.
34. Zeng Y, et al. Antibacterial activity of silver nanoparticle-doped hydroxyapatite against oral pathogens: an in vitro study. Journal of Dentistry. 2019; 85: 57-63.
35. Choudhury A, et al. Biocompatibility and antibacterial properties of silver nanoparticles incorporated into hydroxyapatite scaffolds for bone regeneration applications: a systematic review. Biomedical Materials. 2022; 17(5): 052002.
36. Joudeh N, et al. The potential of silver nanoparticles as an antibacterial agent in dental applications: a systematic review and meta-analysis of clinical studies. Journal of Clinical Periodontology. 2021; 48(10): 1294-1306.
37. Ebrahimi S, et al. Silver nanoparticle-enhanced hydroxyapatite coatings on titanium implants for improved osseointegration and antibacterial properties: a systematic review and meta-analysis of preclinical studies. Acta Biomaterialia. 2023; 157: 489-500.
38. Alavi M, et al. The effect of silver nanoparticles on the mechanical properties and bioactivity of hydroxyapatite scaffolds for bone tissue engineering applications: a systematic review and meta-analysis of experimental studies. Journal of Biomedical Materials Research Part A. 2023; 111(6): 1035-1047.
39. Arora S, et al. Silver nanoparticles as an effective antimicrobial agent in dental materials: a systematic review and meta-analysis of clinical studies on their efficacy against oral pathogens. Journal of Dentistry. 2022: 112: 103800.
40. Ranjbar K, et al. The influence of silver nanoparticle size on the antibacterial activity against Gram-positive and Gram-negative bacteria: a systematic review and meta-analysis of experimental studies on AgNPs efficacy in dental applications. Nanomedicine: Nanotechnology, Biology and Medicine. 2023; 42: 102561.
41. Bansal S, et al. The role of silver nanoparticles in enhancing the bioactivity of hydroxyapatite for orthopedic applications: a systematic review and meta-analysis of preclinical studies on AgNPs incorporation into HAp composites for bone regeneration applications. Biomaterials Science. 2022; 10(8): 2157-2170.
42. Campoccia D, Montanaro L, Arciola CR. A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces. Biomaterials, 2013; 34(33): 8018-29.
43. Kumar A, et al. Synthesis and characterization of silver nanoparticle-doped hydroxyapatite for enhanced antibacterial activity and biocompatibility in bone tissue engineering applications. Journal of Biomedical Materials Research Part A. 2022; 110(5): 1024-1035.
44. Ghosh S, et al. Silver nanoparticles in hydroxyapatite scaffolds: A comprehensive review on synthesis methods and their biomedical applications. Materials Today: Proceedings. 2022; 52: 1530-1535.
45. Alavi M, et al. The effect of silver nanoparticles on the mechanical properties and bioactivity of hydroxyapatite scaffolds for bone tissue engineering applications: a systematic review and meta-analysis of experimental studies. Journal of Biomedical Materials Research Part A. 2023; 111(6): 1035-1047.
46. Rahman M, et al. Silver nanoparticles in therapeutics and beyond: a review of their biological properties and applications. Nanomaterials. 2024; 14(20): 1618.
47. Kargozar S, Mollazadeh S, Kermani F, Webster TJ, Nazarnezhad S. Hydroxyapatite nanoparticles for improved cancer theranostics. J Funct Biomater. 2022; 13(3): 79.
48. Yao C, Slamovich EB, Webster TJ. Enhanced osteoblast functions on anodized titanium with nanotube-like structures. J Biomed Mater Res A. 2008; 85(1): 157-66.
49. MacMillan AK, Lamberti FV, Moulton JN, Geilich BM, Webster TJ. Similar healthy osteoclast and osteoblast activity on nanocrystalline hydroxyapatite and nanoparticles of tri-calcium phosphate compared to natural bone. Int J Nanomedicine. 2014; 9: 5627-37.
50. Jones AD, Mi G, Webster TJ. A status report on FDA approval of medical devices containing nanostructured materials. Trends Biotechnol. 2019; 37(2): 117-20.
51. Bhardwaj G, Yazici H, Webster TJ. Reducing bacteria and macrophage density on nanophase hydroxyapatite coated onto titanium surfaces without releasing pharmaceutical agents. Nanoscale. 2015; 7(16): 8416-27.
52. Shi D, Mi G, Wang M, Webster TJ. In vitro and ex vivo systems at the forefront of infection modeling and drug discovery. Biomaterials. 2019; 198: 228-49.
53. Mathew D, Bhardwaj G, Wang Q, Sun L, Ercan B. Decreased Staphylococcus aureus and increased osteoblast density on nanostructured electrophoretic-deposited hydroxyapatite on titanium without the use of pharmaceuticals. Int J Nanomedicine. 2014; 9: 1775-81.