RESEARCH
www.commprac.com
ISSN 1462 2815

NANO HYDROXYAPPATITE IN 3D POROUS STRUCTURE: FORMATION AND CHARACTERIZATION

Swetha [1], Sangeetha S [2]*, Taniya M [3] and M Sundaram K [4]

[1,2,3,4] Department of Anatomy, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University,
Poonamalle High Road, Velappanchavadi, Chennai.
*Corresponding Author Email: sangeethas.sdc@saveetha.com

DOI: 10.5281/zenodo.12204833

Abstract

Nano hydroxyapatite (nHA), a biocompatible and bioactive material resembling the mineral component of natural bone, exhibits excellent osteoconductivity, promoting bone regeneration and integration with surrounding tissue. Integration of nHA into a three-dimensional (3D) porous structure further enhances its properties, making it ideal for bone tissue engineering, drug delivery systems, and dental implants. In this study, nHA was synthesized using Simulated Body Fluid (SBF) with ion concentrations similar to human blood plasma, maintaining a Ca/P ratio of 2.5. The resulting precipitates were filtered, washed, dried, and calcinated at 900°C to obtain nHA powder. For scaffold preparation, a 10% w/v gelatin solution with 2% w/v xantham gum was mixed with nHA at 10 w/w%. A high foam was induced using acetic acid and sodium carbonate, followed by freeze-drying to create a 3D porous scaffold. The scaffold's morphology and composition were characterized using SEM, EDAX, and FTIR analysis. This study highlights the potential of nHA-loaded gelatin scaffolds in regenerative dentistry and bone tissue engineering due to their biocompatibility, osteoconductivity, and structural properties mimicking the natural extracellular matrix.

Keywords: Nano Hydroxyapatite, 3D Porous Scaffold, Bone Tissue Engineering, Regenerative Dentistry, Gelatin, Osteoconductivity, Biocompatibility, Simulated Body Fluid.

INTRODUCTION

Nano hydroxyapatite (nHA) is a biocompatible and bioactive material that has garnered significant interest in the field of biomaterials due to its resemblance to the mineral component of natural bone (Damiri, Fatimi et al. 2024). Its excellent osteoconductivity promotes bone regeneration and integration with surrounding tissues, making it a vital component in various biomedical applications (Kantharia, Naik et al. 2014). Over the past decades, hydroxyapatite (HA) and its composites with biopolymers have been extensively developed and applied in biomedical fields, particularly in bone tissue engineering, drug delivery systems, and dental implants (Mohd Zaffarin, Ng et al. 2021). Hydroxyapatite, a mineral form of calcium phosphate, closely resembles the composition of natural bone. Its biocompatibility and osteoconductivity make it an ideal material for bone tissue engineering applications. These properties allow it to support bone regeneration effectively by providing a scaffold that cells can adhere to, proliferate, and differentiate on (Munir, Salman et al. 2022). Furthermore, (Jalan, Gayathri et al. 2020, Insuasti-Cruz, Suárez-Jaramillo et al. 2022). Its frequent use in bone grafts, augmentation, and substitution is due to its chemical similarity to bone minerals and its excellent cellular and tissue responses both in vitro and in vivo. The integration of nHA into three-dimensional (3D) porous structures significantly enhances its properties (Ige, Umoru et al. 2012, Chockalingam, Sasanka et al. 2020, Mohanraj, Varshini et al. 2021). These 3D scaffolds mimic the natural extracellular matrix (ECM) found in tissues, providing a supportive structure for cell attachment, proliferation, and differentiation. This makes nHA-loaded scaffolds particularly suitable for regenerative dentistry and bone tissue engineering. In these applications, scaffolds must possess sufficient mechanical strength and stiffness to provide physical support for cell activities and new tissue formation(Silva, Alves et al. 2012). They should also have porous structures with adequate interconnected pores to facilitate the diffusion of nutrients and oxygen, as well as cell ingrowth. Gelatin, a natural polymer derived from collagen, is a major component of the ECM(Divya Sri, Vishnu Priya et al. 2020). It provides mechanical stability to the scaffold and promotes cell adhesion. Gelatin-based scaffolds are advantageous due to their biocompatibility, biodegradability, and ability to form hydrogels that can encapsulate cells and bioactive molecules(Neto and Ferreira 2018). The use of gelatin in combination with nHA helps in creating scaffolds that not only mimic the natural ECM but also support the functional requirements of tissue engineering applications. Incorporating nHA into gelatin-based 3D porous scaffolds enhances the material's properties, making it suitable for various biomedical applications. The 3D porous structure of these scaffolds is crucial as it influences the stress distribution and cell behavior within the scaffold. The pore shape, size, and overall porosity are important factors that affect the scaffold's performance. A well-designed 3D porous scaffold ensures optimal conditions for cell growth and tissue regeneration. To harness the potential of nHA in biomedical applications, it is essential to understand the synthesis and characterization of these materials. In this study, nHA was synthesized using Simulated Body Fluid (SBF) with ion concentrations similar to human blood plasma. The synthesis process involved the addition of calcium chloride (CaCl2) and sodium phosphate (Na2HPO4) to the SBF, maintaining a Ca/P ratio of 2.5 to avoid the precipitation of highly resorbable phases of calcium phosphates(Jalota, Bhaduri et al. 2006). The resulting nHA was then incorporated into a gelatin solution to form a 3D porous scaffold using a foaming technique followed by freeze-drying. The prepared nHA-loaded scaffolds were characterized using various techniques to analyze their morphology, composition, and structural properties. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDAX) were employed to examine the surface morphology and elemental composition of the scaffolds(Birgani 2016). Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups and confirm the chemical structure of the materials. Nano hydroxyapatite-loaded 3D porous gelatin scaffolds represent a promising approach in bone tissue engineering and regenerative dentistry(Nasim, Rajeshkumar et al. 2021). By combining the biocompatibility and osteoconductivity of nHA with the structural and mechanical properties of gelatin, these scaffolds offer a supportive environment for cell growth and tissue regeneration. The synthesis and characterization of these scaffolds are crucial steps in understanding their potential applications and optimizing their performance in clinical settings. This study provides insights into the formation process and properties of nHA-loaded 3D porous scaffolds, highlighting their potential in advancing biomedical applications. While nHA presents significant benefits, its integration into practical biomedical applications involves addressing certain challenges. One key advantage of nHA is its nanoscale size, which enhances its surface area and reactivity, leading to improved interaction with biological tissues(Hutchens 2004). This property is particularly useful in bone tissue engineering where rapid bone in-growth and bonding are desired. However, the synthesis and stabilization of nHA particles can be challenging due to their tendency to aggregate, which can affect the uniformity and performance of the scaffold. The combination of nHA with biodegradable polymers like poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and their copolymers (PLGA) has been explored to enhance the mechanical properties and biological performance of scaffolds. These polymers provide a supportive matrix that degrades over time, coinciding with the formation of new tissue (Çetin 2010). This controlled degradation is crucial for maintaining the structural integrity of the scaffold while promoting natural tissue regeneration. The integration of nHA with these polymers can lead to composite materials that exhibit both excellent mechanical strength and bioactivity. Various techniques have been developed for fabricating 3D porous scaffolds, including freeze-drying, electrospinning, solvent casting, and gas foaming. Each method offers distinct advantages and can be chosen based on the desired properties of the final scaffold. For instance, the freeze-drying technique used in this study is advantageous for creating highly porous structures with interconnected pores, which are essential for nutrient diffusion and cell migration. This method also allows for the incorporation of bioactive molecules and growth factors, further enhancing the scaffold's regenerative capabilities(Kapici).

In regenerative dentistry, nHA-loaded scaffolds are used to repair and regenerate dental tissues, including bone and periodontal ligament(Yazdanian, Rahmani et al. 2021). The scaffolds' ability to mimic the natural ECM and support cellular activities is critical for successful dental regeneration. Additionally, nHA's bioactivity promotes the formation of apatite layers on the scaffold surface, enhancing its integration with natural dental tissues(Sharma, Mathur et al. 2021). This property is particularly beneficial for dental implants, where strong bonding with the surrounding bone is necessary for long-term stability. Extensive in vitro and in vivo studies have demonstrated the efficacy of nHA-based scaffolds in promoting osteogenesis and angiogenesis. In vitro studies typically involve culturing osteoblasts or stem cells on the scaffolds to assess cell adhesion, proliferation, and differentiation. In vivo studies, on the other hand, involve implanting the scaffolds in animal models to evaluate their performance in real physiological environments. These studies are crucial for understanding the biological interactions and ensuring the safety and effectiveness of the scaffolds before clinical application. The future of nHA-based 3D porous scaffolds lies in the development of multifunctional materials that can deliver therapeutic agents, such as antibiotics, growth factors, or anti-inflammatory drugs. These advanced scaffolds can provide localized treatment, reduce infection risks, and promote faster healing. Furthermore, innovations in fabrication techniques, such as 3D printing, can enable the creation of customized scaffolds tailored to individual patient needs. This personalized approach can improve the outcomes of bone regeneration therapies and expand the applications of nHA in other fields, such as cartilage repair and soft tissue engineering. In summary, nano hydroxyapatite-loaded 3D porous scaffolds offer a promising solution for various biomedical applications, particularly in bone tissue engineering and regenerative dentistry. The biocompatibility, osteoconductivity, and structural properties of nHA, combined with the supportive and degradable nature of gelatin and other polymers, make these scaffolds highly effective in promoting tissue regeneration. Continuous advancements in scaffold fabrication, combined with thorough characterization and in vivo studies, are essential for optimizing these materials and translating their potential into clinical practice. This study aims to further explore the formation and characterization of nHA-loaded scaffolds, providing valuable insights into their applications and benefits in the biomedical field(Jampilek and Placha 2021).

ISSN 1462 2815

MATERIALS AND METHODS

Preparation of Bone-Like Hydroxyapatite Nanoparticle (nHA)

Synthesis of Nano Hydroxyapatite:

For the synthesis of nano hydroxyapatite (nHA), Simulated Body Fluid (SBF) with ion concentrations similar to human blood plasma was prepared as reported by Leena et al. (2016). The process began with the preparation of SBF, where the concentrations of calcium chloride (CaCl2, 8.7 M) and sodium phosphate (Na2HPO4, 3.5 M) were adjusted to 3.5 times higher than the reported amounts to maintain a Ca/P ratio of 2.5 and mimic the ionic concentrations of natural human blood plasma. Initially, 0.4935 g of Na2HPO4 (3.5 x 0.141 g) was dissolved in 980 ml of SBF. In a separate solution, 0.9695 g of CaCl2 (3.5 x 0.277 g) was dissolved in the remaining 20 ml of SBF. The CaCl2 solution was then added drop-wise at a rate of 0.5 ml/min to the Na2HPO4 solution under continuous stirring, ensuring thorough mixing and modification of the ionic concentration of the SBF. The pH value of the resulting mixture was adjusted to 7.25. The mixture was incubated for 12 hours to allow for the formation of nHA precipitates. Following incubation, the precipitates were filtered and washed six times with ultrapure water to remove any residual impurities(Shavandi, Bekhit et al. 2015). The washed samples were then dried at 80°C for 24 hours. To study the thermal stability and phase changes of the prepared nHA, the dried samples were calcinated at 900°C for 2 hours in a muffle furnace. This temperature was chosen as nHA is stable up to 900°C and undergoes decomposition beyond this temperature. The final product was crushed using a mortar and pestle to obtain nHA powder suitable for further use.

Preparation of Gradient Porous Nano Hydroxyapatite Loaded 3D Scaffold

Formation of Gelatin Solution:

To prepare the gradient porous nano hydroxyapatite (nHA) loaded 3D scaffold, a gelatin solution was first prepared by dissolving 10% (w/v) gelatin and 2% (w/v) xanthan gum in distilled water. The prepared nHA powder was then added to the gelatin solution at a concentration of 10% (w/w) to form a homogeneous mixture.

Foaming Technique:

A high foam was induced in the gelatin solution using acetic acid and sodium carbonate, each at a concentration of 2%. The nanoparticle-loaded gelatin solution, now with high foam, was rapidly frozen at -80°C and subsequently freeze-dried to create a highly porous 3D scaffold. This method ensured the formation of interconnected porous structures essential for nutrient diffusion and cell migration.

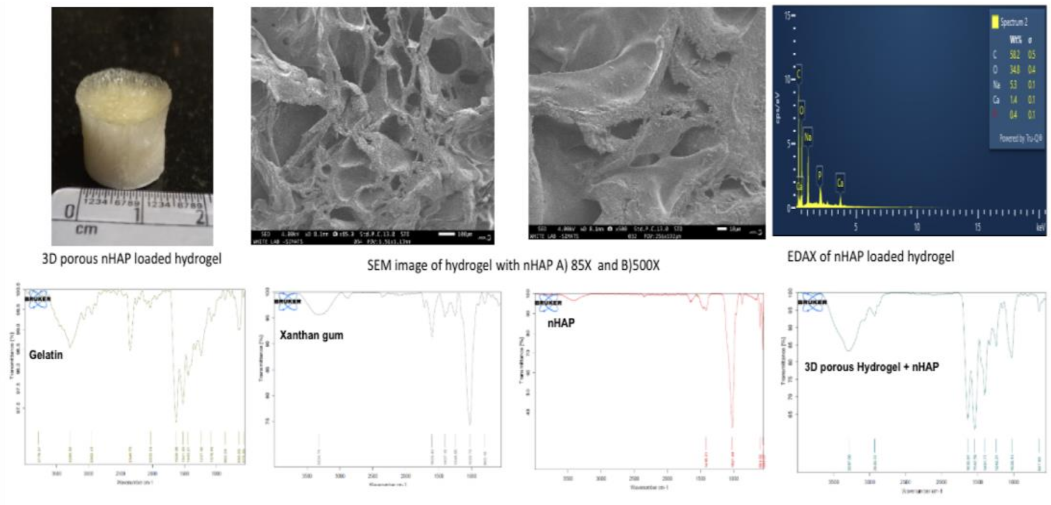
Characterization of the Scaffold:

The morphological characteristics and elemental composition of the nHA and the prepared scaffold were analyzed using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDAX) with a JEOL JSM IT 800. The samples were coated with platinum for 30 seconds prior to imaging. Fourier Transform Infrared Spectroscopy (FTIR) was performed using a Bruker Alpha II model to analyze the chemical structure of the scaffold, with measurements taken from 500 to 4000 cm^-1 at a resolution of 4 cm^-1, averaging 32 scans per sample. Through these methods, the formation and characterization of the nHA-loaded 3D porous scaffold were comprehensively studied, providing insights into its potential

ISSN 1462 2815

applications in biomedical fields such as bone tissue engineering and regenerative dentistry(Abidi and Murtaza 2014).

RESULTS



IR Spectra of A) Gelatin; B) Xanthan gum; C) nHAP and D) 3D porous Hydrogel

DISCUSSION

The formation of nano hydroxyapatite (nHA) in a 3D porous structure represents a significant advancement in the field of biomaterials, particularly for applications in tissue engineering and regenerative medicine(Cai, Wang et al. 2019, BABU and MOHANRAJ 2020). The various methods employed for creating these structures, such as template-based synthesis, gas foaming, and sol-gel methods, each offer distinct advantages. These techniques allow for precise control over the size, shape, and porosity of the resulting nHA structures, which are crucial for their intended applications(Akshaya and Ganesh 2022). The ability to tailor these parameters ensures that the scaffolds can effectively mimic the natural extracellular matrix (ECM), facilitating cell attachment, proliferation, and differentiation. One of the key benefits of using nHA in a 3D porous scaffold is its high surface area and reactivity due to its nanoscale size. This enhances its interaction with biological tissues, promoting rapid bone ingrowth and bonding. However, a significant challenge is the tendency of nHA particles to aggregate, which can affect the uniformity and performance of the scaffold. Overcoming this challenge is critical for ensuring the consistent quality and effectiveness of the scaffold in biomedical applications. The integration of nHA with biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers (PLGA) has been explored to address these challenges. These polymers provide a supportive matrix that degrades over time, aligning with the formation of new tissue. This controlled degradation is essential for maintaining the structural integrity of the scaffold while promoting natural tissue regeneration. The composite materials formed by combining nHA with these polymers exhibit both excellent mechanical strength and bioactivity, making them suitable for a variety of biomedical applications(Zuo, Wei et al. 2017).

The use of gelatin as a biocompatible and biodegradable material in combination with nHA has shown significant potential, especially in regenerative dentistry. The gelatin provides mechanical stability and promotes cell adhesion, while the nHA mimics the mineral component of natural bone, promoting osteoconductivity. The gradient 3D porous scaffold created in this study demonstrates the advantageous properties of both materials, potentially leading to improved outcomes in dental tissue regeneration. In vitro and in vivo studies have shown promising results for nHA-based scaffolds in promoting osteogenesis and angiogenesis. The ability to support cell adhesion, proliferation, and differentiation is crucial for the successful integration of these scaffolds in tissue engineering applications. Additionally, the interconnected porous structure of the scaffolds allows for efficient nutrient and waste exchange, further mimicking the natural ECM and supporting tissue regeneration. The mechanical properties of the nHA scaffolds are also noteworthy. The compressive strength and modulus of the scaffolds are comparable to those of native cancellous bone, indicating their potential for use in bone tissue engineering. In particular, the CPS (cubic pore structure) scaffolds demonstrated the highest compressive strength and modulus, along with enhanced cell metabolism and attachment morphologies. These features are likely due to the larger pore size and smaller curvature of the substrates, which improve cell interaction and metabolic activity. Future research should focus on optimizing the properties of nHA-based scaffolds for specific biomedical applications. The development of multifunctional materials that can deliver therapeutic agents, such as antibiotics, growth factors, or anti-inflammatory drugs, represents a promising direction. Additionally, advances in fabrication techniques, such as 3D printing, can enable the creation of customized scaffolds tailored to individual patient needs, further enhancing the clinical potential of these materials. In conclusion, the formation and characterization of nHA in 3D porous structures hold great promise for various biomedical applications(Vijayalakshmi and Rajeswari 2012). The combination of nHA's bioactivity with the supportive properties of biodegradable polymers offers a versatile platform for developing effective tissue engineering scaffolds. Continuous advancements in this field are essential for translating these materials from the laboratory to clinical practice, ultimately improving patient outcomes in regenerative medicine.

Future Scope

Nano hydroxyapatite in 3D porous structures can be used as scaffolds for tissue engineering, promoting cell attachment, proliferation, and differentiation. The interconnected porous structure allows for nutrient and waste exchange, mimicking the natural extracellular matrix. The future scope of this research includes applications in biomedical engineering, dental and orthopedic implants, biomineralization studies, and the advancement of manufacturing techniques.

Acknowledgement

We extend our sincere gratitude to the Saveetha Dental College and Hospitals for their consent support and successful completion of this work.

References

1) Abidi, S. S. A. and Q. Murtaza (2014). "Synthesis and characterization of nano-hydroxyapatite powder using wet chemical precipitation reaction." Journal of Materials Science & Technology **30**(4): 307-310.

2) Akshaya, A. and K. Ganesh (2022). "Knowledge, prevalence, and impact on social distancing on coronavirus (COVID-19) infection spread in young, adult, and aged population: a survey." ECS Transactions **107**(1): 14063.

3) BABU, B. V. and K. G. MOHANRAJ (2020). "Knowledge, frequency and health hazards of overuse of mobile phone among Chennai population-A questionnaire based survey." International Journal of Pharmaceutical Research (09752366) **12**(4).

4) Birgani, Z. T. (2016). "Bioinorganics: synthetic growth factors for bone regeneration."

5) Cai, Z., et al. (2019). "Large-scale and fast synthesis of nano-hydroxyapatite powder by a microwave-hydrothermal method." RSC advances **9**(24): 13623-13630.

6) Çetin, A. E. (2010). Preparation and characterization of resorbable calcium phosphate based bioceramics, Izmir Institute of Technology (Turkey).

7) Chockalingam, S., et al. (2020). "Role of Bruxism in Prosthetic Treatments-A Survey." Indian Journal of Forensic Medicine & Toxicology **14**(4).

8) Damiri, F., et al. (2024). "Nano-hydroxyapatite (nHAp) scaffolds for bone regeneration: Preparation, characterization and biological applications." Journal of Drug Delivery Science and Technology: 105601.

9) Divya Sri, E., et al. (2020). "Awareness on the role of health care workers in COVID-19-need of the hour." International Journal of Current Research and Review: 122-127.

10) Hutchens, S. (2004). "Synthesis and initial characterization of a calcium-deficient hydroxyapatite-bacterial cellulose composite."

11) Ige, O. O., et al. (2012). "Natural products: a minefield of biomaterials." International Scholarly Research Notices **2012**.

12) Insuasti-Cruz, E., et al. (2022). "Natural biomaterials from biodiversity for healthcare applications." Advanced Healthcare Materials **11**(1): 2101389.

13) Jalan, S., et al. (2020). "Awareness on self isolation to prevent COVID 19 infection among elderly people of Tamilnadu-a survey." International Journal of Current Research and Review: S160-S164.

14) Jalota, S., et al. (2006). "Effect of carbonate content and buffer type on calcium phosphate formation in SBF solutions." Journal of Materials Science: Materials in Medicine **17**(8): 697-707.

15) Jampilek, J. and D. Placha (2021). "Advances in use of nanomaterials for musculoskeletal regeneration." Pharmaceutics **13**(12): 1994.

16) Kantharia, N., et al. (2014). "Nano-hydroxyapatite and its contemporary applications." Bone **34**(15.2): 1.71.

17) Kapici, İ. Development And In Vivo Testing Of Gelatin And Silk-Fibroin Based Calcium Phosphate Bone Cement, Fen Bilimleri Enstitüsü.

18) Mohanraj, K. G., et al. (2021). "Knowledge and Awareness On The Association Between Higher Body Mass Index and Plantar Fasciitis Among Chennain Population-A Survey." Int J Dentistry Oral Sci **8**(03): 1957-1962.

19) Mohd Zaffarin, A. S., et al. (2021). "Nano-hydroxyapatite as a delivery system for promoting bone regeneration in vivo: a systematic review." Nanomaterials **11**(10): 2569.

20) Munir, M. U., et al. (2022). "Synthesis, characterization, functionalization and bio-applications of hydroxyapatite nanomaterials: an overview." International journal of nanomedicine: 1903-1925.

21) Nasim, I., et al. (2021). "Green synthesis of reduced graphene oxide nanoparticles, its characterization and antimicrobial. Properties against common oral pathogens." Int J Dentistry Oral Sci **8**(2): 1670-1675.

22) Neto, A. S. and J. M. Ferreira (2018). "Synthetic and marine-derived porous scaffolds for bone tissue engineering." Materials **11**(9): 1702.

23) Sharma, D., et al. (2021). "Biodegradable and biocompatible 3D constructs for dental applications: Manufacturing options and perspectives." Annals of Biomedical Engineering **49**(9): 2030-2056.

24) Shavandi, A., et al. (2015). "Synthesis of nano-hydroxyapatite (nHA) from waste mussel shells using a rapid microwave method." Materials Chemistry and Physics **149**: 607-616.

25) Silva, T. H., et al. (2012). "Materials of marine origin: a review on polymers and ceramics of biomedical interest." International Materials Reviews **57**(5): 276-306.

26) Vijayalakshmi, U. and S. Rajeswari (2012). "Influence of process parameters on the sol–gel synthesis of nano hydroxyapatite using various phosphorus precursors." Journal of sol-gel science and technology **63**: 45-55.

27) Yazdanian, M., et al. (2021). "Current and advanced nanomaterials in dentistry as regeneration agents: an update." Mini Reviews in Medicinal Chemistry **21**(7): 899-918.

28) Zuo, G., et al. (2017). "Morphology controlled synthesis of nano-hydroxyapatite using polyethylene glycol as a template." Journal of Alloys and Compounds **692**: 693-697.