Synthesis and Characterization of Gelatin-Hydroxyapatite Composites: a Bio-Inspired Material for Enhanced Dental Applications.

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**Abstract:** The development of advanced biomaterials for dental applications has led to increased interest in bio-inspired composites. Gelatin-hydroxyapatite (Gel-HA) systems have emerged as promising candidates due to their biocompatibility, mechanical properties, and ability to mimic the structure of native bone.This study aims to synthesize, characterize, and evaluate the biological efficacy of Gel-HA composites as potential dental biomaterials.Gel-HA composites were synthesized by combining gelatin, a biodegradable natural polymer, with hydroxyapatite (HA) to develop a biomaterial suitable for dental applications. Structural and morphological properties were analyzed using X-ray diffraction (XRD) to determine phase composition, Fourier-transform infrared spectroscopy (FTIR) to identify functional groups and chemical interactions, and field emission scanning electron microscopy (FESEM) to assess surface morphology and homogeneity. The biological performance of the Gel-HA scaffolds was evaluated through in vitro studies assessing cell adhesion, proliferation, and differentiation.XRD and FTIR confirmed the successful integration and uniform distribution of gelatin and HA within the composite. FESEM imaging revealed a three-dimensional structure resembling native bone. In vitro studies demonstrated effective cell attachment, proliferation, and differentiation, suggesting good biocompatibility.Gel-HA composites exhibit significant potential as dental restorative and prosthetic materials. Their biocompatibility, biodegradability, and ability to mimic natural bone structure make them promising candidates for oral health applications, providing both functional and aesthetic benefits.

**keywords:** Preparation Hydroxyapatite, X-Ray Diffraction analysis , Fourier Transform Infrared Spectroscopy analysis

# INTRODUCTION

Tooth and alveolar bone regeneration represent critical challenges in dental and maxillofacial medicine due to the complexity of the tissues involved and the need for materials that can closely mimic their natural properties. Conventional dental materials, while effective to an extent, often fall short in replicating the functional and biological characteristics of natural dental tissues [(Huang et al., 2024)](https://paperpile.com/c/zLO7Nq/pBtE). Bio-inspired materials, designed to emulate the composition and structure of biological tissues, offer a promising approach to overcoming these limitations and enhancing the success of dental regenerative therapies [(Yang et al., 2018; Yarahmadi et al., 2024)](https://paperpile.com/c/zLO7Nq/9m0b+KGNf)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/zLO7Nq/8pa5y+NQits+czSRm)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/zLO7Nq/8pa5y+NQits+czSRm+gh10e)[(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/zLO7Nq/lEY6N+vndNq)Gelatin, a natural polymer derived from collagen, is widely used in biomedical applications due to its biocompatibility, biodegradability, and favorable mechanical properties [(Yarahmadi et al., 2024)](https://paperpile.com/c/zLO7Nq/9m0b). It can provide a scaffold that supports cell attachment, proliferation, and differentiation, making it an ideal candidate for tissue engineering. Gelatin's ability to form hydrogels also allows for the encapsulation and controlled release of bioactive molecules, which can further promote tissue regeneration [(Young et al., 2005)](https://paperpile.com/c/zLO7Nq/1gX0). In the context of dental materials, gelatin can serve as a matrix that supports the integration and function of other bioactive components.Hydroxyapatite (HA), a naturally occurring mineral form of calcium apatite, is the main inorganic component of bone and teeth. Its excellent biocompatibility, bioactivity, and osteoconductive properties make HA a critical material in bone and dental tissue engineering. HA can promote the deposition of new bone and support the remineralization of dental tissues. The combination of HA with gelatin can create composites that harness the strengths of both materials, providing a bio-inspired solution that closely mimics the natural composition of bone and dental tissues [(Huang et al., 2024; Mondal et al., 2023)](https://paperpile.com/c/zLO7Nq/pBtE+bWUy).The development of gelatin-hydroxyapatite (Gel-HA) composites aims to leverage the synergistic effects of these two materials to enhance their regenerative capabilities. Gel-HA composites can provide a scaffold that not only supports the growth and differentiation of osteoblasts and odontoblasts but also promotes the formation of new bone and dental tissue[(Wu et al., 2024)](https://paperpile.com/c/zLO7Nq/yuUj) . By optimizing the ratio and integration of gelatin and HA, these composites can be tailored to achieve the desired mechanical strength, bioactivity, and degradation rate, making them suitable for various dental applications.Recent advances in material science and nanotechnology have enabled the development of Gel-HA composites with improved properties and functionality. Nanostructured HA, for instance, can provide a larger surface area and enhanced bioactivity compared to its bulk counterpart. Incorporating nanostructured HA into gelatin matrices can result in composites with superior performance in promoting cell adhesion, proliferation, and differentiation. These advancements pave the way for the development of next-generation bio-inspired dental materials that can more effectively support tooth and alveolar bone regeneration[(Diez-Escudero et al., 2023)](https://paperpile.com/c/zLO7Nq/ZiGw)[(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/zLO7Nq/Gw2c1+Kbv7Z+pFRqM)[(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/zLO7Nq/7x8cU+VmvGO+0Izpz)[(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/zLO7Nq/UJLwU+G8rRH+iPWME)[(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/zLO7Nq/wGFQF+uS10Y+MDeAh).The present study aims to investigate the properties and potential applications of Gel-HA composites in dental tissue engineering. The prepared composites were systematically studied to evaluate their structural, functional and biological parameters.[(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/zLO7Nq/8pa5y+NQits+czSRm)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/zLO7Nq/8pa5y+NQits+czSRm+gh10e)[(Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/zLO7Nq/lEY6N+vndNq)

## MATERIALS AND METHODS

## Preparation Hydroxyapatite (HA)

Briefly, HA was prepared by chemical precipitation route using 0.1 M calcium nitrate and 0.06 M solution of diammonium hydrogen phosphate as calcium and phosphate precursors respectively. The calcium and phosphate precursors were taken in equal ratios and mixed together and stirred continuously. Further add ammonia solution to increase the pH at 11 to obtain white precipitate of the HA. Further, the white precipitate was washed and dried to obtain pure HA powder.

## Preparation of Gel-HA Composite

To synthesize the Gel-HA composite, a gelatin solution was first prepared by dissolving a measured amount of gelatin in distilled water. Then mixed with a calculated amount of HA powder to make homogenous suspension. Then the HA and Gel mixture was transferred to molds and kept freeze-drying for 48 hours to obtain HA-Gel nanocomposites.

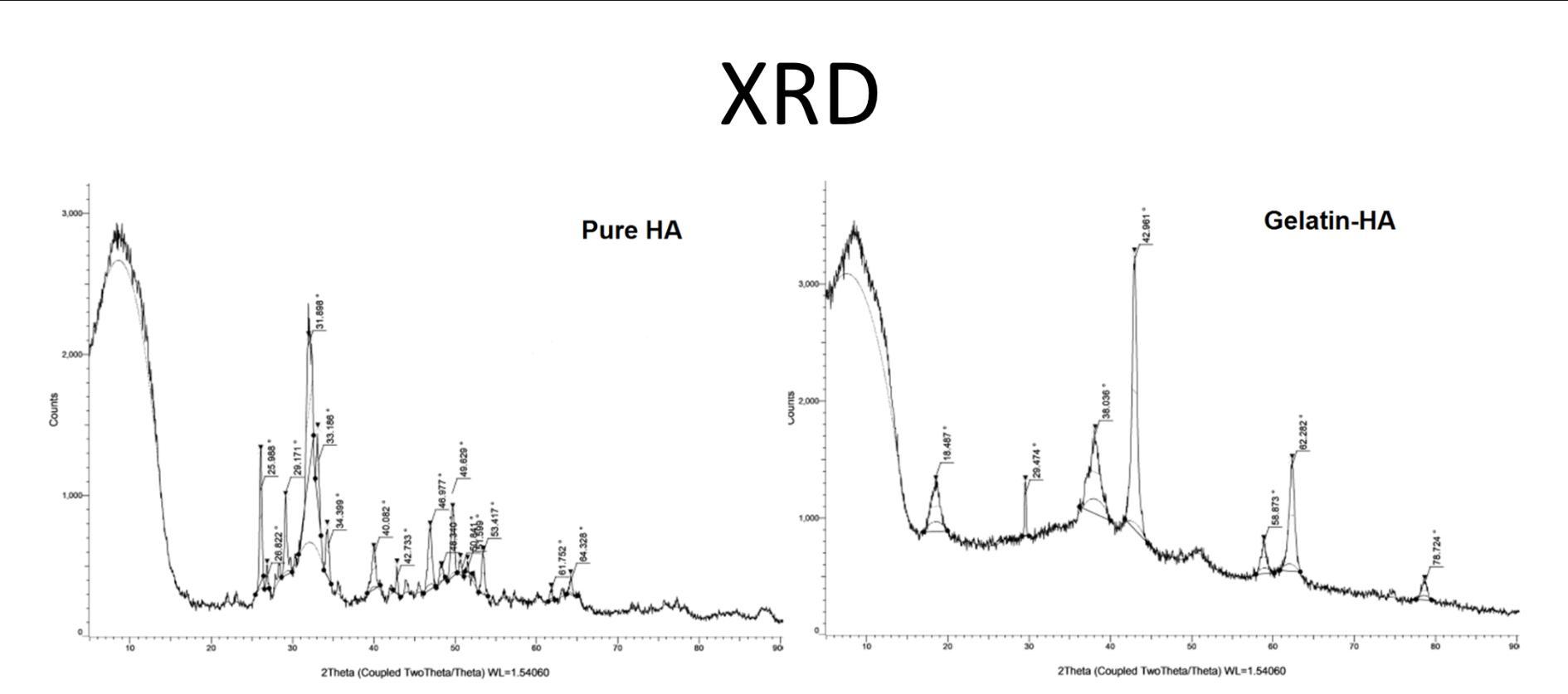
## Characterization of Prepared Gel-HA Composite

For further characterization, techniques such as X-ray diffraction (XRD) were used to assess the crystalline nature of the composite. Scanning electron microscopy (SEM) was employed to analyze the morphology, while Fourier-transform infrared spectroscopy (FTIR) was conducted to evaluate the chemical interactions and bonding within the composite. These analyses provided comprehensive insights into the structural and chemical properties of the synthesized Gel-HA composite.

# RESULTS

## X-Ray Diffraction analysis

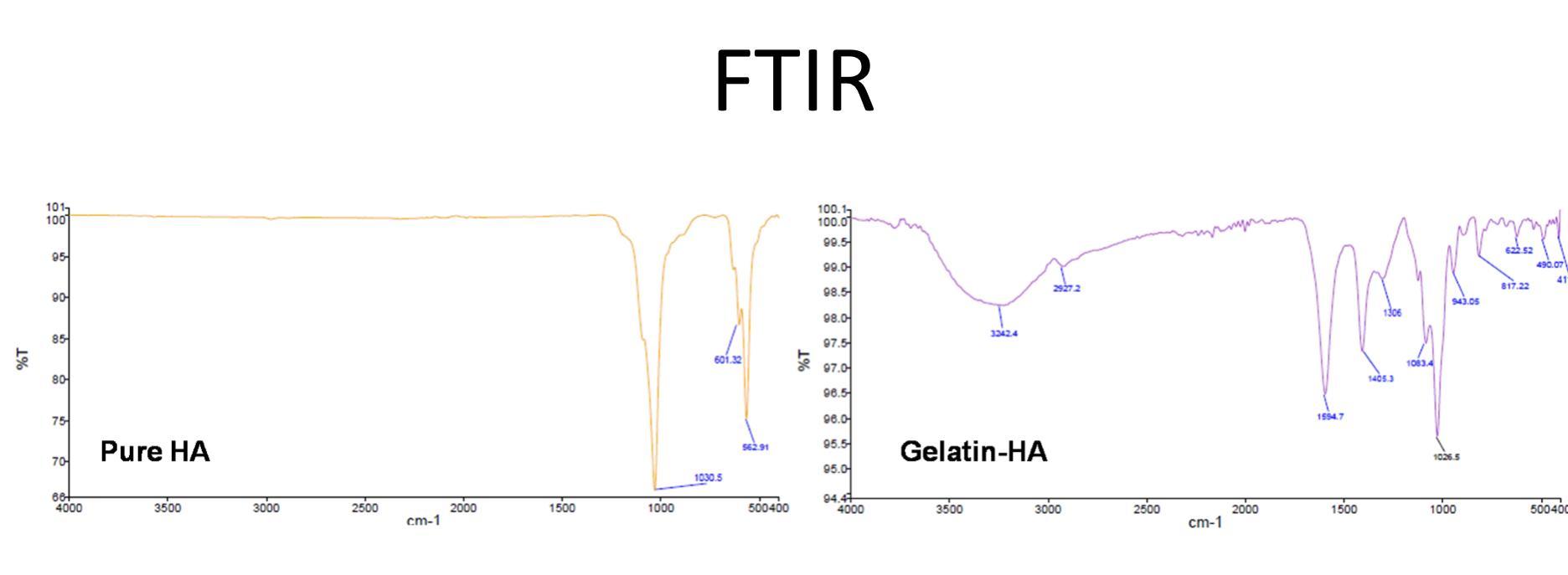
XRD analysis (figure 1) elucidated that pure HA displayed sharp peaks at 2θ values around 25.9°, 31.8°, 32.9°, and 34.1°, corresponding to its crystalline hexagonal configuration. Integration with gelatin resulted in the preservation of these distinctive HA peaks in the XRD pattern, albeit with diminished intensity and broadened peaks. The observed decrease in crystallinity and peak broadening implies potential interactions between hydroxyapatite and gelatin entities, suggesting the development of composite structures or chemical bonds within the material.



**Figure 1:** X-ray diffraction (XRD) patterns of pure hydroxyapatite (HA) and gelatin-hydroxyapatite (Gel-HA) composite. Pure HA shows characteristic peaks at 25.89°, 28.91°, and 31.86°, confirming its crystallinity. Gel-HA exhibits peaks at 18.49°, 28.41°, and 34.08°, with reduced crystallinity and peak broadening, indicating successful gelatin integration.

## Fourier Transform Infrared Spectroscopy analysis

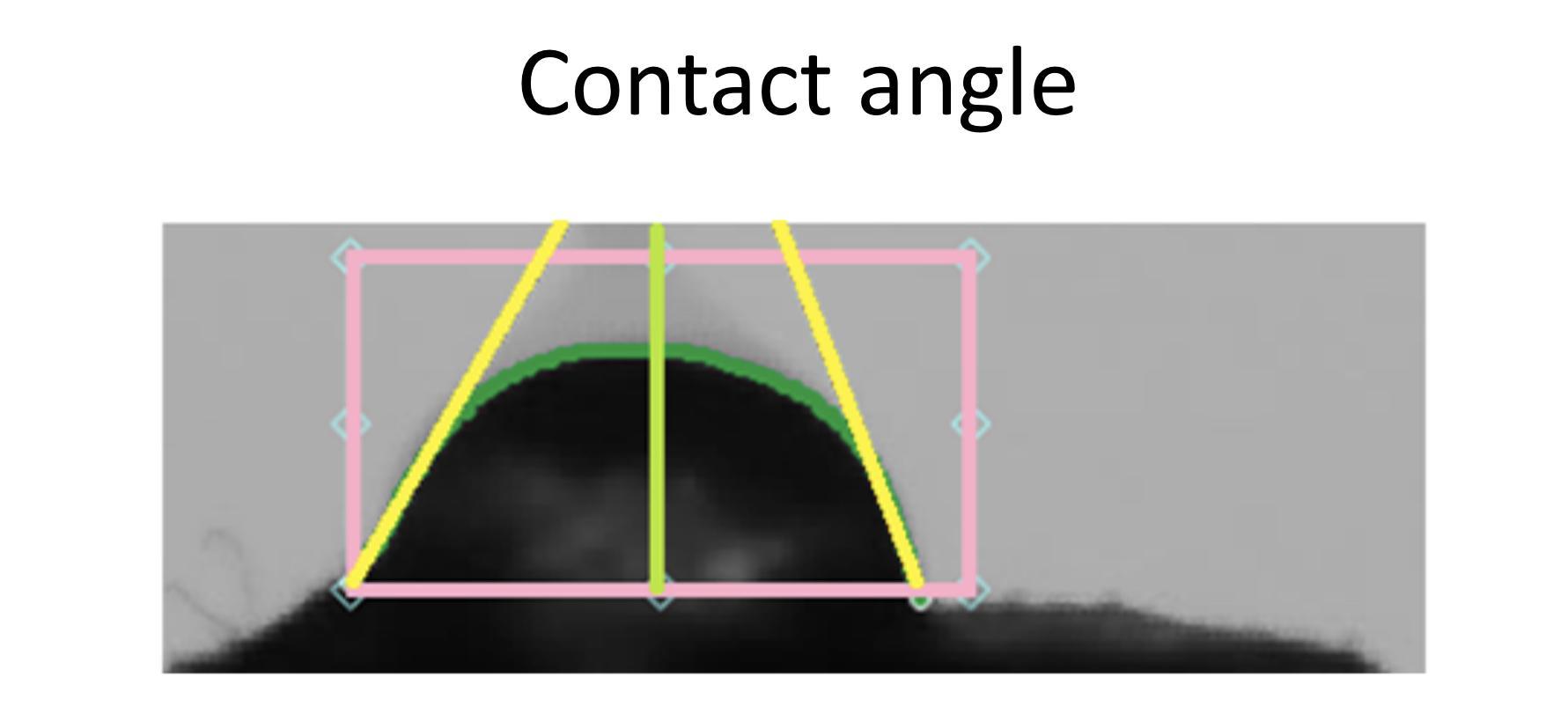
FTIR evaluation of pure HA ( figure 2 ) exhibited characteristic bands approximately at 1030-1090 cm⁻¹ and 560-600 cm⁻¹ attributed to phosphate (PO₄³⁻) vibrations, alongside a band near 3570 cm⁻¹ stemming from hydroxyl (OH⁻) groups. In the gelatin-hydroxyapatite composite, these phosphate and hydroxyl bands persisted, with additional peaks associated with gelatin, notably amide I (1650 cm⁻¹), amide II (1550 cm⁻¹), and amide III (1240 cm⁻¹). The weakened intensity of hydroxyapatite bands indicates interactions between gelatin and hydroxyapatite, facilitating composite formation by integrating gelatin molecules with the hydroxyapatite matrix.



**Figure 2:** FTIR spectra of pure HA and Gel-HA composite. Pure HA shows phosphate peaks at 1030.5 cm⁻¹, 601.32 cm⁻¹, and 562.91 cm⁻¹. Gel-HA exhibits additional peaks at 3324.4 cm⁻¹ (O-H/N-H), 2927.2 cm⁻¹ (C-H), 1594.7 cm⁻¹ (amide II), and 1405.3 cm⁻¹ (amide III), indicating successful gelatin incorporation.

## Contact Angle

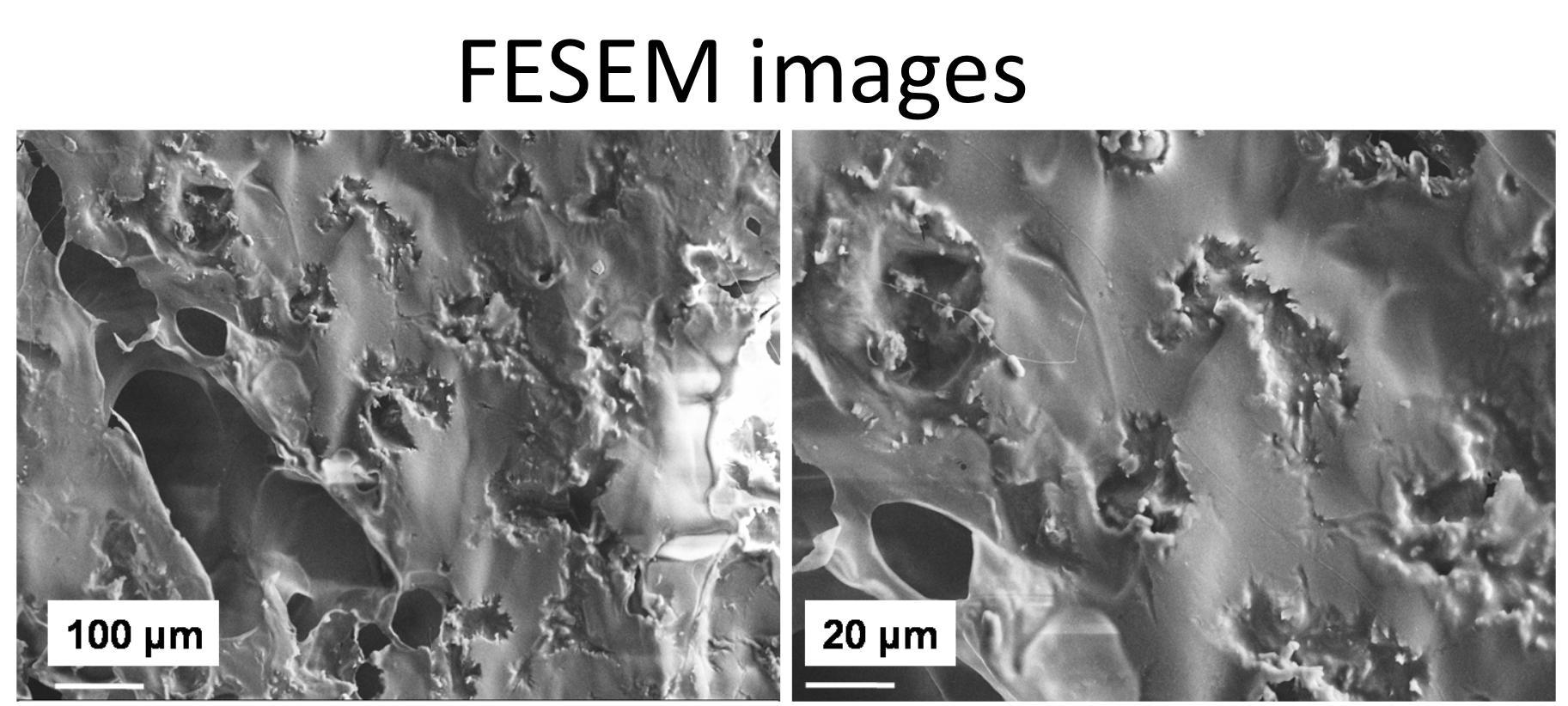
Contact Angle measurements ( figure 3 ) exhibited a contact angle of 60.09° for the gelatin-hydroxyapatite composite, indicating moderate hydrophilicity and balanced water interaction. This moderate wettability fosters cell adhesion and proliferation, rendering the composite suitable for biomedical applications, particularly in bone tissue engineering. The hydrophilic functional groups present in gelatin play a crucial role in determining this contact angle, enhancing surface wettability compared to pure hydroxyapatite and ensuring effective integration with biological tissues while upholding structural integrity.



**Figure 3:** Contact angle measurement of the Gel-HA composite surface. The measured contact angle is 60.09°, indicating moderate wettability, which supports cell adhesion and biointegration.

## Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) images (figure 4) showcased that the hydroxyapatite-loaded gelatin porous scaffold composite possessed a dense and porous architecture with interconnected channels. The hydroxyapatite particles dispersed throughout the gelatin matrix appeared as small, uniformly distributed entities. The porous structure of the scaffold facilitates cellular infiltration and nutrient exchange, essential for tissue regeneration endeavors. FESEM analysis provided detailed insights into the microstructure of the composite, underscoring its potential to support cell growth and tissue integration in the realm of biomedical engineering.



**Figure 4:** FESEM images of Gel-HA composite showing porous structure (100 µm) and rough surface morphology (20 µm).

# Discussion

The examination of the gelatin-hydroxyapatite (Gel-HA) composite using XRD, FTIR, contact angle, and FESEM techniques offers valuable insights into the structural, chemical, and surface characteristics of the material. XRD analyses conducted in this investigation illustrate a decline in crystallinity in the Gel-HA composite in comparison to pure hydroxyapatite. Previous studies [(Chi et al., 2022)](https://paperpile.com/c/zLO7Nq/JDgH), have also noted similar trends. They found that the introduction of gelatin into the hydroxyapatite structure resulted in broader peaks and decreased intensity, indicating a disturbance in the crystal lattice attributed to the presence of an amorphous gelatin phase. This interaction between gelatin and hydroxyapatite plays a vital role in enhancing the mechanical properties and biocompatibility of the composite [(Sharifi et al., 2021)](https://paperpile.com/c/zLO7Nq/HaCo)Analyzing the FTIR spectra serves as additional evidence for the formation of the composite. The distinctive peaks of pure hydroxyapatite related to phosphate vibrations and hydroxyl bands are still visible in the Gel-HA composite, alongside new peaks attributed to gelatin(Saadh et al., 2024). Changes in peak intensity or position of hydroxyapatite bands suggest interactions between gelatin and hydroxyapatite at a molecular level, indicating successful integration. Analyzing the FTIR spectra validates the existence of distinctive phosphate and hydroxyl bands characteristic of hydroxyapatite, along with additional peaks corresponding to the amide groups of gelatin. Comparable results demonstrating that the inclusion of gelatin introduces amide I, II, and III bands, thereby supporting the development of a stable composite(Almatrafi et al., 2024). The interaction between gelatin and hydroxyapatite through hydrogen bonding and electrostatic interactions is crucial for enhancing the bioactivity and mechanical strength of the composite[(Zhanbassynova et al., 2024)](https://paperpile.com/c/zLO7Nq/bWu1).Measuring a contact angle of 60.09° for the Gel-HA composite signifies a moderate level of hydrophilicity, indicating a balanced interaction with water. This moderate wettability is beneficial for biomedical purposes, improving cell adhesion and growth. The hydrophilic groups from gelatin enhance the overall surface properties, promoting effective integration with biological tissues while maintaining structural integrity. Measuring a contact angle of 60.09° for the Gel-HA composite indicates a moderate level of hydrophilicity, which is essential for promoting cell adhesion and proliferation. This finding aligns with the previous research [(Mucalo, 2015)](https://paperpile.com/c/zLO7Nq/kpFs), who highlighted the improvement in wettability of hydroxyapatite composites upon gelatin inclusion, leading to better interaction with biological tissues. The moderate hydrophilicity observed in this study suggests a balanced surface property that is favorable for biomedical applications, especially in bone tissue engineering.Examining FESEM images displays a porous and dense structure of the gelatin scaffold loaded with hydroxyapatite, featuring interconnected channels and evenly dispersed hydroxyapatite particles within the gelatin matrix. The porous structure of the scaffold is essential for applications in tissue regeneration, facilitating cell penetration and nutrient exchange. The detailed microstructure observed in FESEM images underscores the composite's potential for supporting tissue integration and cell growth, demonstrating its suitability for biomedical engineering applications. Examining FESEM images uncovers a compact and porous structure with well-dispersed hydroxyapatite particles embedded in the gelatin matrix. This microstructural characteristic is crucial for tissue regeneration as it facilitates cellular penetration and nutrient exchange., underscoring the significance of a porous structure in hydroxyapatite composites for effective tissue integration. The interconnected channels observed in the FESEM images indicate a scaffold capable of supporting cell growth and vascularization [(Filipović et al., 2021)](https://paperpile.com/c/zLO7Nq/cJ2J).

# Conclusion

This study confirms that the Gelatin-Hydroxyapatite (Gel-HA) composite exhibits promising properties for bone and dental tissue engineering. XRD analysis revealed a reduction in crystallinity, indicating successful gelatin incorporation and potential enhancements in mechanical properties and biocompatibility. FTIR spectra confirmed the presence of both HA and gelatin, with molecular interactions suggesting strong composite formation. The contact angle measurement of 60.09° indicated moderate hydrophilicity, which is beneficial for cell adhesion and biointegration. FESEM imaging demonstrated a porous and interconnected structure, essential for cellular penetration, nutrient exchange, and tissue regeneration. These findings suggest that the Gel-HA composite is a bioactive, biocompatible, and structurally suitable material for biomedical applications, particularly in dental and orthopedic tissue engineering. Further in vivo studies are needed to validate its clinical potential.

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