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Alginate-hydroxyapatite scaffolds: A comprehensive characterization study

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ABSTRACT

Introduction: Alginate has garnered significant attention in regenerative dentistry for its biocompatibility, mechanical strength, and controlled biodegradability. The incorporation of hydroxyapatite enhances its ability to mimic the dentin extracellular matrix, promoting cellular adhesion, proliferation, and mineralization. This study aims to comprehensively assess the structural, chemical, and biological properties of Alg-HA scaffolds to evaluate their potential for dentin regeneration.

Methods: Alginate-hydroxyapatite (Alg-HA) scaffolds were synthesized by dissolving sodium alginate (2 % w/v) in distilled water, followed by the incorporation of hydroxyapatite (HA) synthesized via chemical precipitation using calcium nitrate tetrahydrate and ammonium phosphate. The composite solution was homogenized through stirring and ultrasonication before being freeze-dried to fabricate porous scaffolds. Characterization was performed using X-ray Diffraction (XRD) to confirm crystallinity, Fourier Transform Infrared Spectroscopy (FTIR) to verify functional group interactions, and Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray Analysis (EDAX) to analyze morphology and elemental composition. In vitro degradation studies were conducted in simulated body fluid (SBF) to assess scaffold stability by measuring mass loss over time, with additional pH monitoring and SEM analysis for morphological changes. Hemocompatibility was evaluated through hemolysis assays, comparing scaffold-incubated blood samples to positive and negative controls.

Results: XRD analysis confirmed the successful incorporation of HA within the alginate matrix, highlighting characteristic HA peaks and alginate's amorphous nature. FTIR analysis validated the composite formation through phosphate-carboxylate interactions. SEM imaging revealed a porous, interconnected structure with embedded HA particles, facilitating cell attachment and proliferation. EDAX confirmed the presence of calcium, phosphorus, and oxygen as primary constituents. In vitro degradation studies showed controlled degradation, with 80 % mass loss by day 3, indicating the composite's suitability for gradual tissue replacement. Hemocompatibility tests revealed minimal hemolysis (<2 %), confirming the composite's excellent blood compatibility.

Conclusion: The findings emphasize the potential of Alg-HA scaffolds for dentin regeneration. Their porous architecture, combined with embedded HA, enhances mechanical stability while providing essential biochemical cues for cell proliferation and mineralization. The demonstrated hemocompatibility ensures safe application in direct blood contact, reducing immune responses and promoting tissue integration. Compared to previous studies, this research offers a more in-depth understanding of the relationship between porosity, mineralization, and cellular behavior. Alginate-hydroxyapatite scaffolds exhibit excellent structural, chemical, and biological properties, making them promising candidates for regenerative dentistry. With excellent degradation, hemocompatibility, and ability to support cellular functions, these scaffolds hold significant potential for clinical applications, with further optimization paving the way for broader medical adoption.

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1. Introduction

Dentin, a mineralized tissue that forms the majority of the tooth structure, plays a crucial role in maintaining tooth integrity and function. It is characterized by a network of tubules extending from the pulp chamber to the dentin-enamel junction (DEJ).[1] These tubules are encased by hyper-mineralized peritubular dentin, with intertubular dentin filling the spaces in between. The intertubular dentin is composed of a collagen fibril matrix reinforced with nanoscale apatite crystals. This complex microstructure endows dentin with unique mechanical properties, which vary spatially, with hardness and strength decreasing closer to the pulp. Such variations are significant in restorative dentistry, where the mechanical performance of dentin under stress is a critical consideration.[2]

Recent advancements in regenerative endodontics have highlighted the potential of dental pulp stem cells (DPSCs) and other dental stem cells in regenerating the dentin-pulp complex. DPSCs, known for their high proliferative and differentiation capacity, have shown promise in regenerating dentin, bone, and even pulp tissue when supported by appropriate scaffolds.[3,4] An ideal scaffold must provide mechanical support and promote cell attachment, proliferation, and differentiation.[5]

Alginate (Alg), a naturally occurring polysaccharide, has gained attention as a scaffold material due to its biocompatibility, low toxicity, and ability to form hydrogels in divalent cations. When combined with hydroxyapatite (HA), a mineral closely resembling the inorganic phase of dentin, the resulting composite scaffolds exhibit enhanced mechanical properties and bioactivity.[6,7] These scaffolds provide a conducive environment for cell infiltration and mineralization, making them suitable for tissue engineering applications.[8] This study aims to explore the potential of alginate-hydroxyapatite scaffolds in supporting dentin regeneration by evaluating their structural, mechanical, and biological properties, focusing on their ability to promote the growth and differentiation of dental pulp stem cells.

2. Materials & methods

2.1. Preparation of alginate solution

A 2 % (w/v) sodium alginate solution was prepared by dissolving 2 g of sodium alginate (Sigma Aldrich, India) powder in 100 mL of distilled water. The mixture was stirred continuously at room temperature until a clear, homogeneous solution was obtained.[9] Sterilization was achieved by autoclaving at 121 °C for 15 min or filtering through a 0.22 μm filter.

2.2. Synthesis of hydroxyapatite (HA)

Hydroxyapatite was synthesized via the chemical precipitation method. A 0.5 M calcium nitrate tetrahydrate [$Ca(NO_3)_2{\cdot}4H_2O$] solution (Sisco Research Laboratories Pvt. Ltd. SRL, India) and a 0.3 M ammonium phosphate [$(NH_4)_2HPO_4$] solution (Merck, India) were prepared as precursors. The calcium nitrate solution was added dropwise to the ammonium phosphate solution under constant stirring while maintaining the pH at 10 using ammonia (Sisco Research Laboratories Pvt. Ltd. SRL, India). The resulting precipitate was collected by filtration, washed with distilled water, and dried at 100 °C overnight. The dried precipitate was calcined at 600 °C for 2 h to obtain pure HA powder.

2.3. Preparation of hydroxyapatite-alginate composite

Hydroxyapatite powder (1 % w/v) was gradually added to the 2 % alginate solution under continuous stirring to ensure even dispersion. The mixture was stirred for several hours until homogeneous. Ultrasonication was used to enhance dispersion if necessary. The final composite solution was filtered through a 0.22 μm filter to maintain sterility.[10]

2.4. Scaffold fabrication via freeze-drying

The hydroxyapatite-alginate composite solution was poured into molds and rapidly frozen at −80 °C. The frozen samples were then transferred to a freeze-dryer. Primary drying was conducted under a vacuum to sublimate ice, followed by secondary drying to remove bound water.[11] The resulting porous scaffolds were stored in sterile conditions for further analysis.

2.5. Characterization

The scaffolds were characterized using various techniques to evaluate their structural, chemical, and biological properties. X-ray Diffraction (Unique *D8* diffractometer family platform, Germany) was employed to analyze the crystallographic structure and phase composition of hydroxyapatite within the scaffold. Fourier Transform Infrared Spectroscopy (Alpha II, Platinum-ATR, Bruker Optics, Germany) was used to identify functional groups and confirm chemical interactions between alginate and hydroxyapatite. Scanning Electron Microscopy was utilized to examine the surface morphology and porosity of the scaffolds, providing insights into their microstructural features essential for cell attachment and proliferation. Energy Dispersive X-ray Analysis (EDAX) was performed in conjunction with SEM (SEM - JEOL-JSM-IT800 Electron Microscopy, Japan) to determine the elemental composition and verify the uniform distribution of hydroxyapatite within the scaffold matrix.[10]

2.6. Measurement of scaffold degradation rate

The scaffold's degradation behavior was assessed through in vitro degradation studies in simulated body fluid (SBF) to evaluate its stability over time. The degradation rate of the scaffold was evaluated through in vitro degradation testing by immersing the scaffold in a physiological environment and measuring mass loss over time. Initially, scaffolds of known dry weight (W0W_0W0) were recorded and then incubated in phosphate-buffered saline (PBS) at 37 °C to simulate body conditions. In some cases, enzymatic degradation using lysozyme may have been employed to mimic biological degradation. The medium was refreshed periodically to prevent accumulation of degradation byproducts. At specific time intervals (e.g., 1, 3, 7, 14, and 28 days), the scaffolds were removed, rinsed with deionized water, dried to a constant weight, and weighed (Initial weight – Wi, after drying, it is noted as Wt).[12] The degradation percentage was calculated using the formula:

Additionally, pH monitoring of the degradation medium was performed to assess changes due to byproducts, while morphological changes were examined using SEM analysis, and mechanical integrity was evaluated over time. This methodology ensures accurate assessment of scaffold degradation, addressing the concern about how degradation was measured.

2.7. Hemocompatibility of the scaffold

The biocompatibility test for our scaffold was assessed by mixing 50 μL of blood with 950 μL of double-distilled water for the positive control and mixing 50 μL of blood with 950 μL of phosphate-buffered saline (PBS) for the negative control. The scaffold samples were then incubated with the blood solution, and the rate of hemolysis was evaluated by comparing the absorbance of the test samples against the positive and negative controls.[13] Together, these analyses provided comprehensive insights into the scaffold's structural, chemical, and biological properties, highlighting its suitability for dentin regeneration applications.

3. Results

3.1. XRD analysis

The X-ray Diffraction (XRD) analysis of the alginate-hydroxyapatite composite confirms the successful incorporation of hydroxyapatite into the alginate matrix. The XRD pattern exhibits characteristic peaks of hydroxyapatite at 2θ values around 25.9°, 31.8°, 32.9°, and 34.1°, indicative of its crystalline hexagonal structure. However, reduced peak intensity and slight broadening are observed, attributed to the

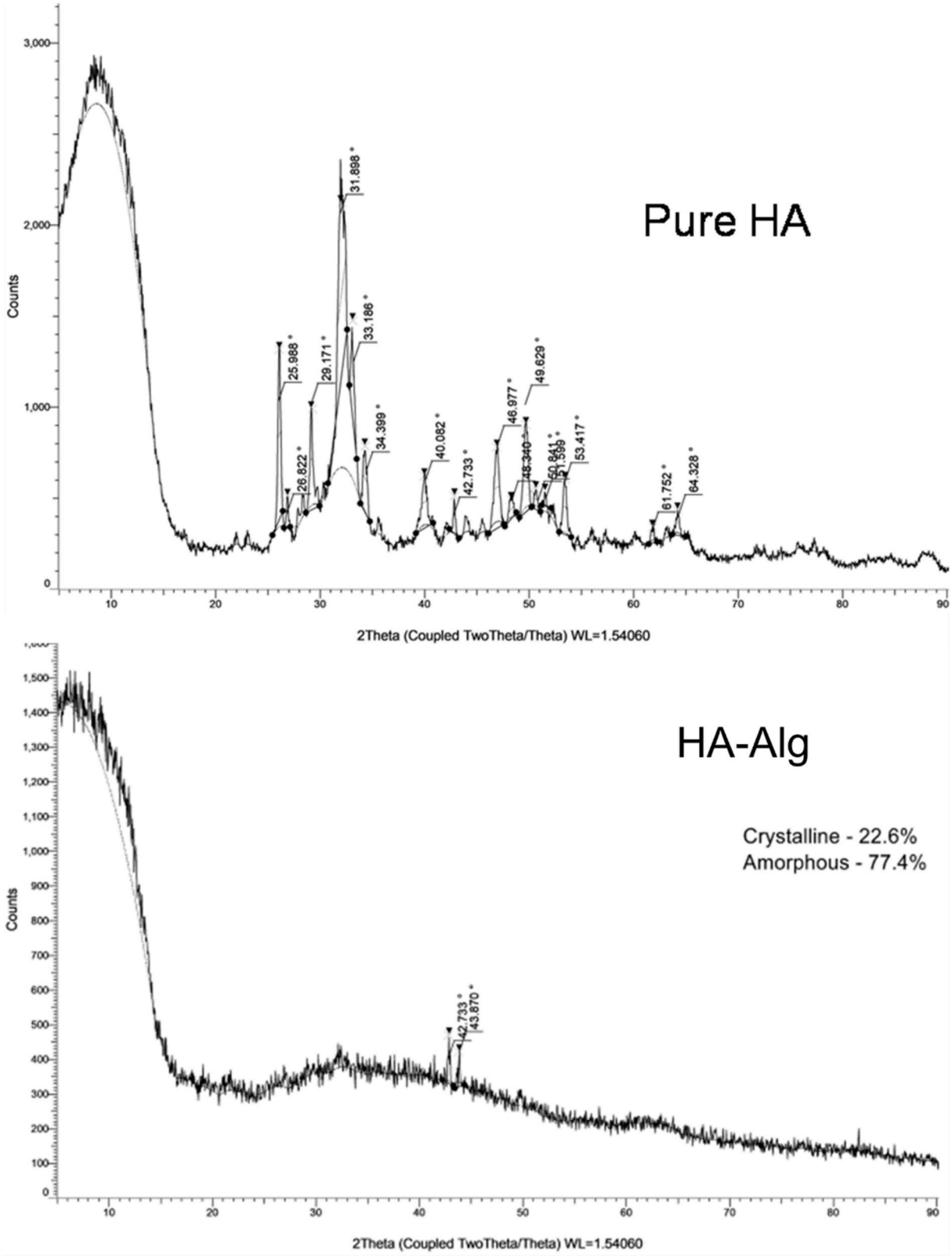

Fig. 1. XRD analysis of Pure HA and alginate-hydroxyapatite composite.

amorphous nature of alginate, which disperses the hydroxyapatite particles. Quantitative analysis reveals that the composite consists of 22.6 % crystalline phase and 77.4 % amorphous phase, with a broad hump at lower 2θ angles confirming the amorphous alginate's dominance (Fig. 1). This dual-phase composition balances the bioactive properties and mechanical strength provided by hydroxyapatite with the structural disorder and porosity of alginate, making the composite ideal for tissue engineering applications like dentin regeneration, where both osteoconductivity and high porosity are crucial.

3.2. FTIR analysis

The Fourier-transform infrared spectroscopy (FTIR) analysis of the alginate-hydroxyapatite composite confirms the successful integration of both components. The FTIR spectrum displays characteristic absorption bands of hydroxyapatite, including prominent peaks at 1030-1090 cm^{-1} and 560-600 cm^{-1}, corresponding to phosphate (PO_4^{3-}) group vibrations, along with a band near 3570 cm^{-1} for hydroxyl (OH^-) groups. Additionally, the spectrum shows alginate-specific bands, with asymmetric and symmetric stretching of carboxylate (COO^-) groups at approximately 1600 cm^{-1} and 1400 cm^{-1}, respectively (Fig. 2). The coexistence of these bands indicates effective interaction between alginate's carboxylate groups and hydroxyapatite's phosphate groups, forming a stable composite structure. This successful integration highlights the composite's potential for tissue engineering and regenerative medicine, leveraging hydroxyapatite's bioactivity and alginate's biocompatibility and flexibility to enhance cell attachment and growth.

3.3. SEM analysis

The SEM images provide valuable insights into the surface morphology and microstructure of the alginate-hydroxyapatite (HA) scaffolds. The lower magnification reveals a porous morphology with well-distributed, interconnected pores (Fig. 3A). This network of pores is crucial for facilitating nutrient and waste exchange, as well as supporting cell infiltration and tissue ingrowth. At higher magnification, the scaffold's surface exhibits a rough and flaky texture, which significantly increases the surface area, offering numerous potential sites for cell attachment (Fig. 3B). Additionally, small dots observed on the surface indicate the presence of hydroxyapatite (HA) particles embedded within

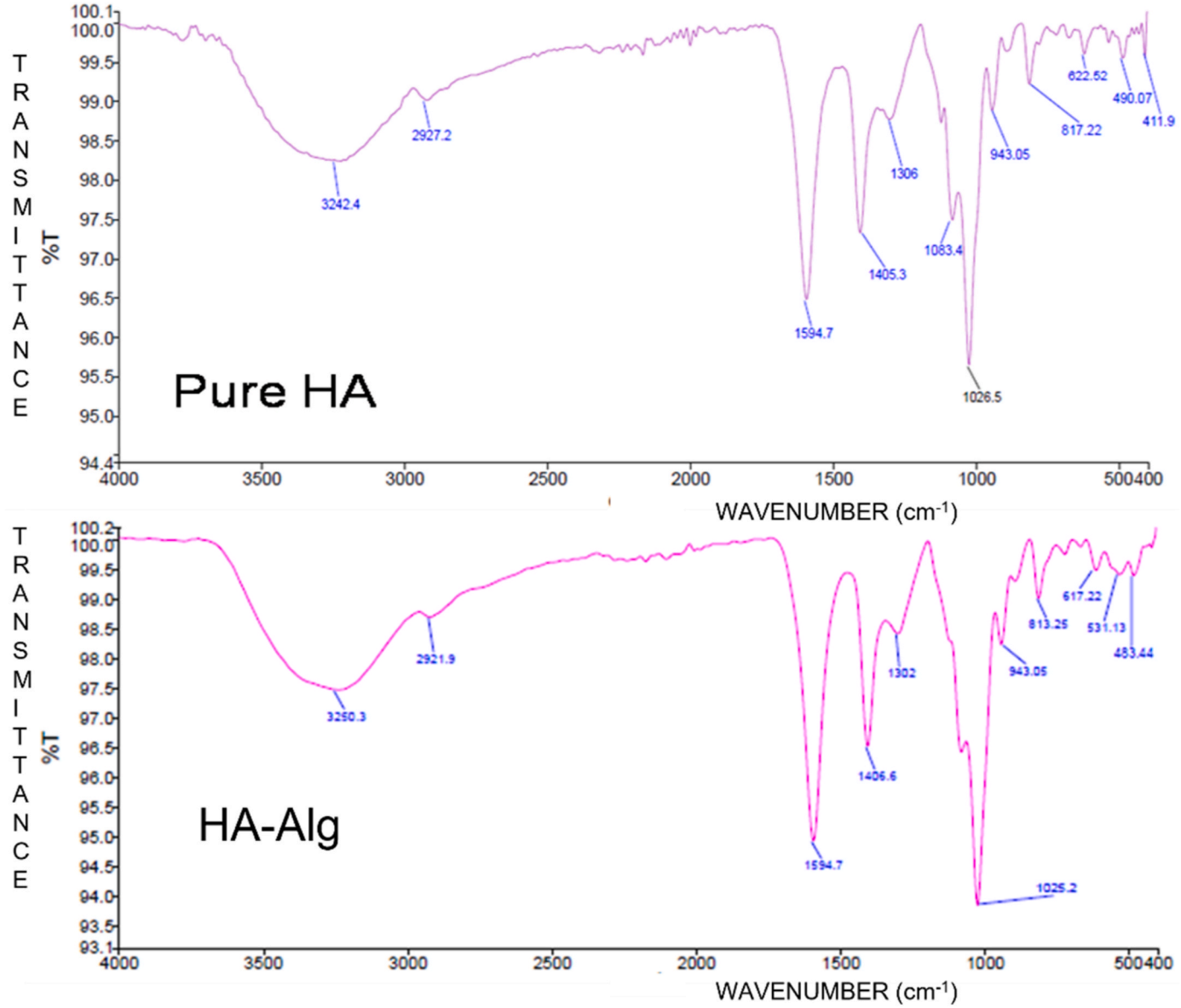

Fig. 2. FTIR analysis of the alginate-hydroxyapatite composite where X-axis represents wavenumber (cm^{-1}) and Y-axis represents Transmittance (%).

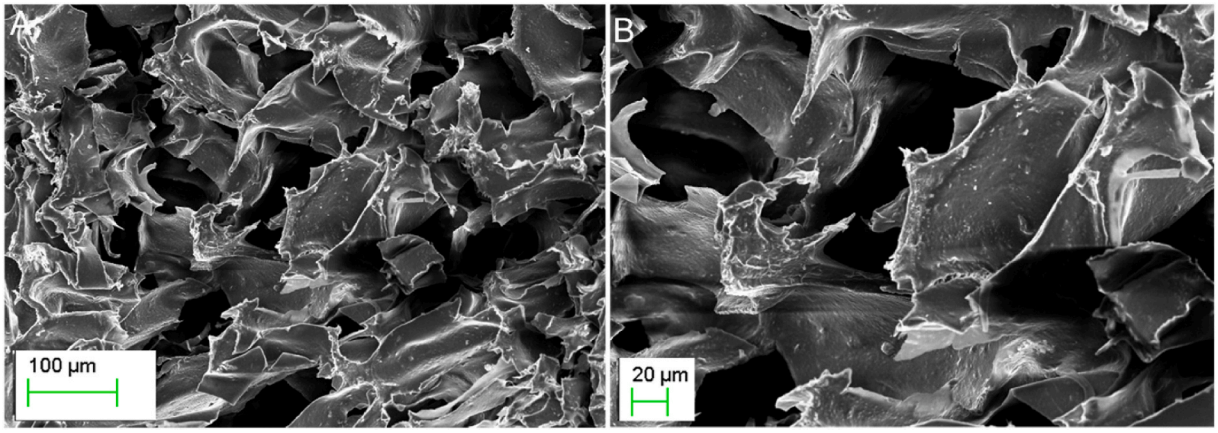

Fig. 3. shows the SEM analysis of the alginate-hydroxyapatite composite.

the alginate matrix. This distribution of HA enhances the scaffold's structural and biochemical properties, making it suitable for mimicking the extracellular matrix and promoting cellular activities such as adhesion, proliferation, and mineralization.

3.4. EDAX analysis

The EDAX spectrum complements the SEM analysis by providing the elemental composition of the alginate-HA scaffold. Peaks corresponding to calcium (Ca), phosphorus (P), and oxygen (O) confirm the presence of hydroxyapatite, which is primarily composed of calcium phosphate. The spectrum also indicates minor elements such as magnesium (Mg), sodium (Na), and chlorine (Cl), which could either originate from the scaffold components or residual salts from the synthesis process. The Ca/P ratio in the scaffold aligns with the stoichiometry of hydroxyapatite, reinforcing the successful integration of mineral phases (Fig. 4). The presence of carbon (C) corresponds to the alginate matrix, while the oxygen peaks reflect contributions from both alginate and hydroxyapatite. This elemental analysis validates the composite's chemical composition, ensuring its suitability for dentin regeneration.

3.5. In vitro degradation

The in vitro degradation analysis of the alginate-hydroxyapatite (HA-Alg) composite compared to pure alginate (Alg) over three days reveals distinct differences in their degradation rates. At day 0, both materials remain intact with 0 % degradation. By day 1, pure alginate exhibits a degradation rate of approximately 40 %, whereas the HA-Alg composite shows a higher degradation rate of around 50 %. By day 3, the degradation rate of pure alginate increases to nearly 50 %, while the HA-Alg composite reaches approximately 80 %, indicating that the presence of hydroxyapatite influences the degradation behavior, leading to a higher but more controlled degradation rate over time (Fig. 5). These findings highlight that hydroxyapatite incorporation effectively slows the degradation of alginate, offering improved structural integrity and making the composite more suitable for tissue engineering applications

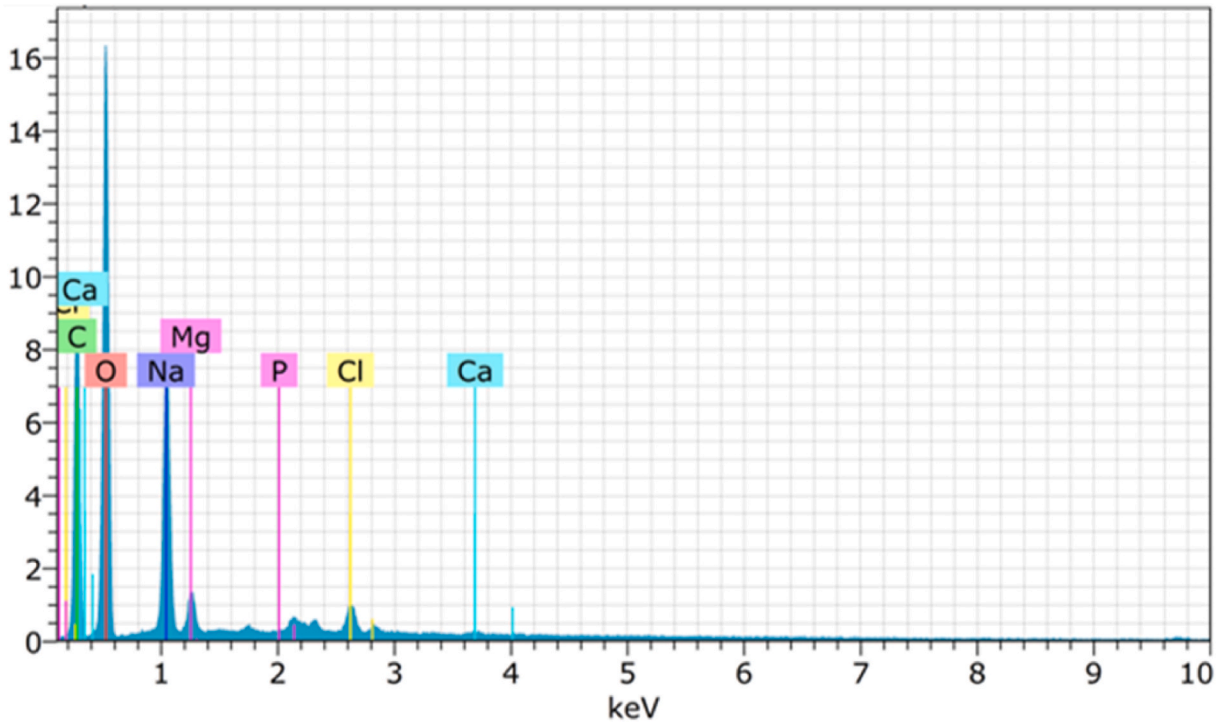

Fig. 4. shows the EDAX analysis of the alginate-hydroxyapatite composite.

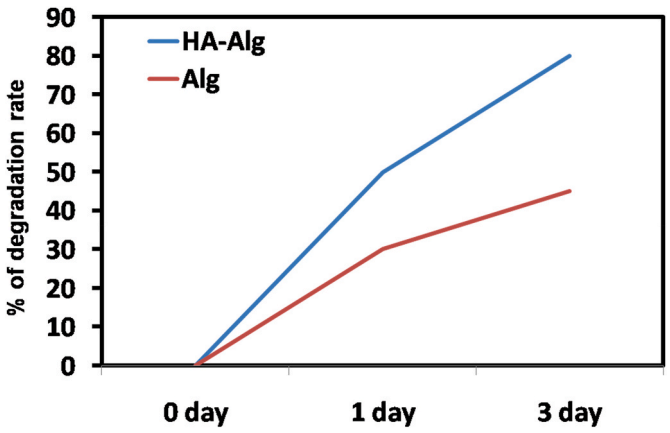

Fig. 5. In vitro degradation of the alginate-hydroxyapatite composite.

where gradual degradation is essential for supporting tissue regeneration.

3.6. Hemocompatibility

The hemolysis evaluation of the Alginate (Alg) and Hydroxyapatite-Alginate (HA-Alg) composite, as shown in the bar graph, compares the percentage of hemolysis induced by both materials. The Y-axis represents the hemolysis percentage, ranging from 0 % to 3 %, while the X-axis distinguishes between the Alg and HA-Alg groups. The results show that both materials exhibit hemolysis rates below 2 %, with HA-Alg showing a slightly higher rate, though still well below the critical threshold (Fig. 6). This suggests that both materials are highly compatible with blood, making them suitable for applications in contact with blood, such as bone grafts and tissue engineering scaffolds. The low hemolysis rates of these composites indicate minimal risk of immune response or complications, supporting their potential for safe integration and functionality in medical implants and treatments. These findings highlight the promising biocompatibility of Alginate and Hydroxyapatite-Alginate composites for biomedical use.

4. Discussion

The use of alginate-hydroxyapatite (HA) composite scaffolds for dentin regeneration represents a promising approach in tissue engineering, combining the advantages of both materials to provide a biocompatible and structurally supportive environment for tissue repair.

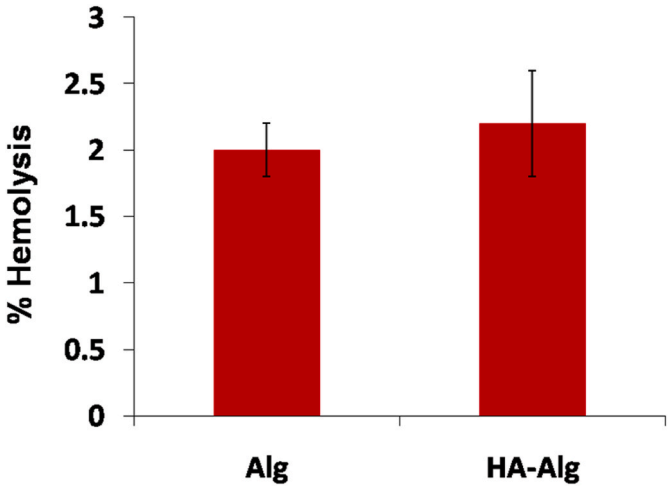

Fig. 6. Hemocompatibility of the alginate-hydroxyapatite composite.

Previous studies have explored the use of various biomaterials for dentin regeneration, including natural polymers like collagen and synthetic materials like polylactic-co-glycolic acid (PLGA), but few have successfully combined biodegradable polymers like alginate with bioceramics like hydroxyapatite to achieve the dual benefits of bioactivity and structural stability.[14,15] This study builds on these earlier efforts by focusing specifically on the alginate-HA combination, leveraging its unique properties to overcome some of the challenges seen in prior research.

One of the key advantages of alginate-based scaffolds, as discussed in the current study, is its biodegradability. Alginate's ability to gradually degrade in vivo, allowing for natural tissue replacement, has been well-established in earlier studies.[16,17] Alginate's hydrophilic nature facilitates cell adhesion and proliferation, crucial elements for promoting tissue regeneration. However, while pure alginate scaffolds have been reported in prior studies, their rapid degradation rates have posed challenges in maintaining the structural integrity necessary for effective tissue engineering. Some studies have incorporated hydroxyapatite into alginate matrices to slow degradation and enhance mechanical properties.[18–20] This study, however, offers a more detailed exploration of the degradation kinetics of alginate-HA composites in aqueous environments, showing that incorporating HA slows degradation and ensures long-term scaffold stability. The controlled degradation rate observed in this study makes the composite scaffold a more reliable option for dentin regeneration, where prolonged scaffold integrity is critical.

The combination of alginate and hydroxyapatite has also been shown to enhance the scaffold's mechanical strength and its osteoconductive properties, which are important for both dentin regeneration and bone tissue engineering. While hydroxyapatite has been widely used in bone tissue engineering due to its ability to mimic the mineralized extracellular matrix of bone, its integration into alginate-based scaffolds for dentin regeneration is relatively novel.[21,22] This study adds to the growing body of knowledge by showing that the hydroxyapatite phase within the composite enhances the scaffold's mechanical stability, an essential feature for providing structural support to regenerating tissues. Previous studies have focused on the incorporation of HA into various polymer matrices, but the ability to tailor the hydroxyapatite content for specific applications in dentin regeneration, as shown in this study, highlights a unique contribution.[23,24] The balance between biodegradability and structural stability achieved through the careful modulation of the alginate-to-hydroxyapatite ratio sets this study apart from previous research.

In terms of physicochemical properties, the FTIR analysis presented in this study further confirms the successful formation of the alginate-HA composite. The characteristic absorption bands of hydroxyapatite, such as those corresponding to phosphate vibrations (1030-1090 cm^{-1}) and bending (560-600 cm^{-1}), in combination with the alginate bands (1600 cm^{-1} for asymmetric COO^- stretching and 1400 cm^{-1} for symmetric COO^- stretching), provide strong evidence of the integration of both materials into a single, homogeneous scaffold. While FTIR spectra of alginate-HA composites have been reported in previous studies, this study provides a more comprehensive analysis of how these bands correlate with the composite's mechanical and biological properties, such as enhancing cell adhesion and promoting mineralization, which is key for effective dentin regeneration.[25,26]

Our results demonstrate that both alginate and the alginate-HA composite induce hemolysis rates well below the acceptable threshold of 5 %, indicating excellent hemocompatibility. The slightly higher hemolysis rate observed for the alginate-HA composite, while still minimal, may be attributed to the presence of hydroxyapatite particles, which could introduce minor surface roughness or ionic interactions affecting the red blood cell membranes. In comparison to other biomaterials, such as synthetic polymers or ceramic-based scaffolds, which may require surface modifications to improve their hemocompatibility, the alginate-HA composite demonstrates inherent blood compatibility.[27,28]

Another unique aspect of this study is the exploration of the scaffold's porosity and phase composition. The importance of porosity in tissue engineering, particularly for ensuring effective nutrient and waste exchange, has been well-documented in previous research. However, the study also highlights the significance of hydroxyapatite in mimicking the mineralized matrix of dentin, which is essential for supporting odontoblast activity and subsequent dentin formation. Previous studies have not thoroughly investigated the interaction between the scaffold's porosity and mineralization characteristics and their influence on cell behaviour in the context of dentin regeneration. This study bridges that gap by providing a comprehensive analysis of both porosity and mineral composition, offering deeper insights into how the alginate-HA scaffold supports tissue regeneration through a combination of structural and biochemical cues.

5. Limitations of the study

The present study provides a detailed characterization of alginate-hydroxyapatite scaffolds; however, certain aspects remain to be explored. While the mechanical and physicochemical properties were analysed, aspects such as ion release, and bioactivity in simulated body fluids require further investigation. A comparative analysis with existing biomaterials or commercially available scaffolds was not performed, which could help establish its relative performance. Furthermore, challenges related to large-scale fabrication and clinical translation were not addressed. Despite these limitations, the findings provide valuable insights into the fundamental properties of alginate-hydroxyapatite scaffolds, forming a strong basis for future research aimed at optimizing their application in dentistry.

6. Conclusion

In summary, this study offers several novel contributions to the field of dentin regeneration using alginate-hydroxyapatite scaffolds. By focusing on the dual properties of biodegradability and structural stability, the study provides a more refined understanding of how these composites can be tailored for specific tissue engineering applications. Furthermore, the detailed physicochemical analysis, including FTIR and degradation rate assessments, adds valuable insights into the behaviour of these materials in vivo. The unique combination of hydroxyapatite's bioactivity and the structural properties of alginate, along with their ability to promote both tissue regeneration and scaffold stability, sets this study apart from previous research in this area. This work lays the foundation for further research into the optimization of alginate-hydroxyapatite composites for dentin regeneration, offering potential for clinical applications in regenerative dentistry.

Ethical clearance

Ethical clearance is not applicable as this is invitro study.

Patient's consent

Not Applicable.

Source of funding

Nil.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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