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Characterization of Polyvinyl Alcohol/Pectin Hydrogel Incorporated with Hydroxyapatite Bone Scaffold Applications

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ABSTRACT

Various biomaterials have been explored as a result of the growing demand for efficient bone regeneration techniques, driven by the increasing prevalence of bone fractures. Pectin is a natural biomaterial with polysaccharide structure that have capability enhances biocompatibility and promotes cell adhesion and proliferation. Additionally, hydroxyapatite, a naturally occurring mineral in bone, was included to provide osteoconductive properties and improve the mechanical strength of the hydrogel scaffold. This study aims to develop and characterize a new hydrogel composed of polyvinyl alcohol (PVA), pectin, and hydroxyapatite (HA) for bone regeneration applications. Gelation was initiated using calcium chloride and glutaraldehyde to facilitate in situ crosslinking. Experimental evaluations included injectability, gelation time, surface morphology (SEM analysis), elemental composition (EDX analysis) and chemical structure (FTIR analysis), The gelation times were recorded as 1 hour for the control (PVA/pectin), 1.5 hours for PVA/pectin/5% HA, and 2 hours for PVA/pectin/10% HA. SEM analysis revealed an interconnected porous structure essential for cell infiltration and nutrient diffusion. FTIR and EDX analyses confirmed the successful incorporation of HA into the hydrogel matrix. Among the formulations, the 5% HA hydrogel exhibited the most balanced characteristics in terms of gelation time, and structural properties. These findings contribute to the advancement of hybrid hydrogel scaffolds, providing insights into innovative solutions for bone defect treatments in clinical applications.

INTRODUCTION

Bone fractures have become a growing concern in modern society, with their prevalence increasing due to a variety factors including falls, road traffic accidents, sports injuries and age-related diseases like osteoporosis. These factors significantly impact bone integrity, where it requires the use of bone scaffold implants. Existing research has provided valuable insights into biological and mechanical processes underlying bone healing, significant gaps remain in understanding the optimal conditions and materials needed to enhance scaffold performance. Bone scaffolds serve as a supportive framework, providing a stable environment to facilitate the repair process and promote the natural regeneration of bone tissue (Lee et. al., 2022).

Scaffolds play a crucial role in supporting bone regeneration by providing a structure for cells to grow and integrate with the surrounding tissue. The 3D design of scaffolds allows them in guiding cells through biochemical cues, such as signalling molecules and physical cues, such as structural properties like

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pore size and stiffness. These cues are vital for directing cell behaviour, promoting adhesion, proliferation, differentiation and ultimately influencing tissue remodelling (Zhang and King 2020). Scaffolds, in general, act as matrices designed to anchor cells, promoting their growth and proliferation, providing rigidity to the implanted tissue and creating space for vascularization. Additionally, scaffolds are designed to transport, store and release active factors that stimulate distinct cellular reactions, that support the treated region's mechanical and structural integrity (Perez-Puyana et. al., 2020). However, the ideal scaffold material, design and characteristics are still under investigation particularly in terms of their ability to mimic natural bone tissue and promote efficient healing

Conventional polymer hydrogels have shown limitations in biomedical applications due to their weak mechanical strength and stability, which limits their ability to function effectively as scaffolds. These shortcomings restrict their application in load-bearing tissues or environments requiring durability. To address these issues, a hybrid polymer is developed by combining polyvinyl alcohol (PVA) and pectin, which alters the chemical properties of the hydrogel through the enhancement in mechanical characteristics and stability. Additionally, conventional polymer hydrogels have been known in some rare cases that it can trigger the allergic reactions due to its natural origin.

Pectin, a complex anionic polysaccharide naturally found in the cell walls and fruits of higher plants, has emerged as an ideal biomaterial for bone regeneration applications due to its intrinsic properties and versatility. As a biocompatible, biodegradable, and FDA-approved biopolymer, pectin offers significant advantages in tissue engineering. It is composed of poly-α-D-galacturonic acid residues linked by α(1−4) glycosidic bonds, with partially esterified methyl carboxyl groups that enhance its functional properties, such as water-holding capacity, viscosity, and gel formation. These properties make pectin an excellent mineralization substrate for bone tissue engineering, as it can regulate apatite size, an important factor in mimicking the natural mineral composition of bone (Sultana, 2023). The ability of pectin to undergo gelation through the introduction of calcium ions further supports its application in hydrogel systems. This gelation process results in an "egg-box" structure, which provides a stable matrix capable of immobilizing bioactive components or cells within the gel. This structure not only facilitates cell attachment and proliferation but also enhances the delivery and retention of bioactive molecules essential for bone regeneration. Moreover, pectin's nontoxicity, diverse chemical composition, and high stability contribute to its ability to support long-term applications in biomedical settings (Feng et al 2022).

Pectin also plays a role in immunomodulation, an essential consideration in pathological conditions, including bone defect repair. By regulating the body's immune response to foreign materials, pectin helps create a favourable environment for bone healing while minimizing adverse reactions. This property is particularly valuable in scaffold-based tissue engineering, where the immune system's response can significantly impact the success of the implanted material. The combination of pectin's ability to form hydrogels, regulate apatite mineralization, and modulate immune responses makes it a highly effective additive in hydrogel-based scaffolds (Akshata et al., 2023).

Another significant limitation of polymer hydrogels is their poor cell orientation and adhesion, which are critical for effective scaffold performance, as these factors influence cell behavior, tissue growth and regeneration. To overcome this problem, hydroxyapatite (HA), a naturally occurring mineral known for its excellent biocompatibility, is incorporated into PVA/Pectin matrix, thereby enhancing the biological properties of the scaffold, promoting better cell attachment, proliferation and differentiation. This hybrid approach combining PVA, pectin and HA aim to create a more robust, biocompatible and effective scaffold for biomedical applications, addressing the key shortcomings of conventional polymer hydrogels

MATERIALS AND METHOD

Preparation of Polyvinyl Alcohol/pectin with Hydroxyapatite Hydrogel

The fabrication of the hydrogel begins with preparing stock solutions of the required materials. A 10% polyvinyl alcohol (PVA) solution was prepared by dissolving PVA powder in deionized water, which then was heated to 95°C and stirred until fully dissolved. Similarly, a 1% pectin solution was prepared by dissolving pectin powder in deionized water, ensuring complete dissolution. Additionally, a 2% calcium chloride ($CaCl_2$) solution was prepared by dissolving anhydrous calcium chloride in deionized water. These stock solutions are essential for forming the polymeric matrix and crosslinking the hydrogel. The next step involves mixing the PVA and pectin solutions in a 2:1 ratio, ensuring the formation of a stable composite structure. In this study, 4 mL of PVA solution was combined with 2 mL of pectin solution. The ratio of 2:1 between PVA and pectin was used to balance the hydrogel's mechanical strength and hydrophilicity, with PVA providing the primary structural integrity and pectin contributing to the biodegradability and bioactivity of the scaffold.

Once the PVA-Pectin solution was homogeneously mixed, hydroxyapatite (HA) was added in varying concentrations of 0%, 5%, and 10% by weight to explore the impact of HA on the hydrogel's properties. In this case, 0g (control), 0.3g (5%), and 0.6g (10%) of hydroxyapatite are added to the composite solution, allowing for the evaluation of the role of HA in enhancing osteogenic potential and mechanical stability. The inclusion of HA was critical for mimicking the inorganic component of bone, promoting bioactivity, and potentially improving the mechanical properties of the hydrogel.

Next, 2 mL of calcium chloride solution was added to the PVA-Pectin-HA mixture, ensuring a PVA:Pectin:$CaCl_2$ ratio of 2:1:1. This specific ratio was important for optimizing the crosslinking process, where calcium chloride acts as a crosslinking agent that interacts with the hydroxyl groups in PVA and the carboxyl groups in pectin, leading to the formation of a stable polymer network. The 2:1:1 ratio ensures that the crosslinking density is sufficient to enhance the mechanical strength and stability of the hydrogel while maintaining its desired porosity and swelling behaviour.

To further stabilize the hydrogel network, 0.1% glutaraldehyde solution was added to the mixture. Glutaraldehyde, at a 25% concentration, was used in very small quantities as a crosslinking agent to strengthen the polymer matrix by forming covalent bonds between the polymer chains, improving mechanical stability and reducing the degradation rate of the hydrogel.

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Finally, the mixture was poured into a petri dish, where it is left at room temperature to undergo gelation. During this phase, the hydrogel solidifies into a stable, crosslinked structure. The gelation process is critical for the formation of a well-defined hydrogel network, and the controlled setting at room temperature allows for the gradual formation of a homogeneous gel that retains its shape and integrity. Once gelation completed, the hydrogel samples were ready for further characterization and analysis.

Characterization of PVA/Pectin with Hydroxyapatite Hydrogel.

The characterization of the PVA/Pectin with HA hydrogel was conducted to evaluate its structural, chemical, and functional properties, which are crucial for its potential application in bone regeneration.

Morphology Surface Testing

The morphological surface analysis of the PVA/Pectin hydrogel with hydroxyapatite (HA) was conducted using a Hitachi TM 3000 tabletop scanning electron microscope (SEM). Five samples were prepared for this analysis which are one PVA/Pectin hydrogel as control, two samples of PVA/Pectin hydrogel with 5% HA, and two samples of PVA/Pectin hydrogel with 10% HA. The hydrogels were freeze-dried prior to SEM analysis to remove water content while preserving the structural integrity of the polymer matrix, as water could disrupt the vacuum environment required for SEM imaging and can cause image distortion. After freeze-drying, the samples were cut into uniform dimensions of 5 mm × 5 mm with a thickness of 5 mm to ensure consistent analysis.

Elemental Composition Identification

Elemental composition of the PVA/Pectin hydrogel with hydroxyapatite (HA) was analysed using Energy Dispersive X-ray (EDX) spectroscopy coupled with the Hitachi TM 3000 tabletop scanning electron microscope (SEM). Five samples were prepared for this analysis which are one PVA/pectin hydrogel as control, two samples of PVA/pectin hydrogel with 5% HA, and two samples of PVA/pectin hydrogel with 10% HA. The method of preparing samples for EDX analysis is same as SEM as it underwent the same analysis machine.

EDX analysis was performed during SEM imaging, where the sample was bombarded with an electron beam, causing the elements within the sample to emit characteristic X-rays. These X-rays were captured by the EDX detector, which provides information on the elemental composition of the hydrogel, specifically the presence of HA within the composite. By analysing the energy levels of the X-rays, the elemental distribution and composition of the samples were identified, offering insights into the incorporation of HA and its effect on the hydrogel's overall structure.

Confirmation of Functional Groups Formed

To confirm the functional groups formed within the hydrogel matrix, Fourier Transform Infrared (FTIR) spectroscopy was employed using a PerkinElmer FTIR spectrometer Three samples were prepared for analysis which are one PVA/pectin hydrogel as control, one PVA/Pectin hydrogel with 5% HA, and one PVA/Pectin hydrogel with 10% HA. Prior to FTIR analysis, the hydrogel samples were freeze-dried to remove the water content. Freeze-drying is crucial as it preserves the hydrogel's structural integrity by preventing any moisture-related interference during the analysis. The samples were cut into 1 cm × 1 cm pieces with a thickness of 1 cm to ensure uniformity and sufficient material for FTIR analysis.

RESULT AND DISCUSSION

Gelling Time of PVA/Pectin Hydrogel with Hydroxyapatite

The gelling time of the hydrogel increases with the addition of hydroxyapatite (HA) content due to the interaction between the polymer network and the inorganic particles. The PVA/Pectin hydrogel without hydroxyapatite, serving as the control, exhibited the shortest gelling time of 1 hour. This is because the crosslinking process occurs more freely in the absence of additional fillers, allowing faster formation of the hydrogel network. When 5% hydroxyapatite is incorporated, the gelling time increases to 1.5 hours due to the interaction between HA particles and the polymer chains. The HA particles act as physical barriers, slowing down the mobility of polymer chains and thus delaying the crosslinking process. With 10% hydroxyapatite, the gelling time further extends to 2 hours as the higher concentration of HA particles amplifies these interactions, creating more resistance to the chain mobility and requiring more time for the network to form completely (Jalageri et al., 2022). Additionally, hydroxyapatite's inherent hydrophilicity may attract water molecules (Ma and Wang, 2015), altering the hydration dynamics and further slowing down the gelling process (Usinskas et al., 2016). These findings demonstrate that increasing HA content systematically delays gelling time due to the altered physicochemical interactions within the hydrogel system. Fig 1 displays the hydrogels after certain period of gelling time that placed in Petri dishes.

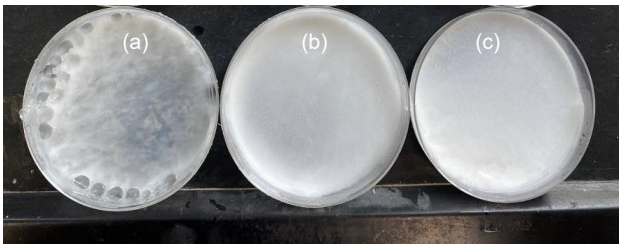

Fig 1 Gelling time took by the samples to become hydrogel (a) PVA/pectin hydrogel (1 hour), (b) PVA/pectin hydrogel with 5% hydroxyapatite (1.5 hours) and (c) PVA/pectin hydrogel with 10% hydroxyapatite (2 hours)

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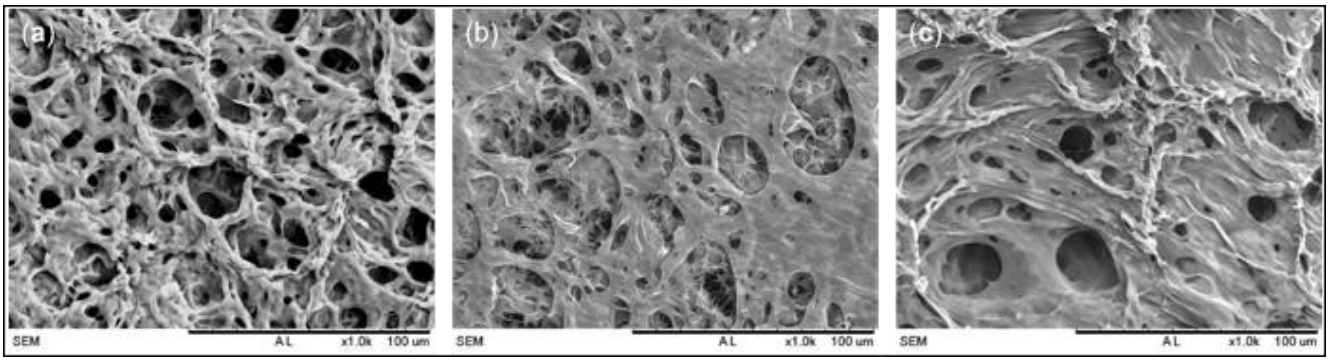

Fig 2 Morphological surface analysis using Scanning Electron Microscope (**a**)PVA/pectin hydrogel (**b**)PVA/pectin hydrogel with 5% hydroxyapatite (**c**)PVA/pectin hydrogel with 10% hydroxyapatite

Morphology Surface Testing

The surface morphology of the hydrogels, as observed under Scanning Electron Microscopy (SEM), reveals the influence of hydroxyapatite (HA) content on porosity and interconnectivity as shown in Fig 2. The PVA/Pectin hydrogel without HA, as the control of the study, exhibits a highly porous structure with excellent interconnectivity. This is due to the intrinsic properties of the polymer network, where phase separation during hydrogel formation creates well-defined pores (Feng et al., 2022). The high porosity and interconnectivity are crucial for tissue engineering applications, as they allow efficient cell infiltration, nutrient diffusion, and waste removal.

With the incorporation of 5% HA, the porosity is noticeably reduced, and the interconnectivity diminishes. The reduced porosity can be attributed to the presence of HA particles, which occupy space within the polymer matrix, disrupting the formation of uniform pores. Furthermore, the interaction between HA particles and polymer chains leads to a denser and less open network structure. The diminished interconnectivity occurs because the particulate phase interferes with the formation of continuous pore channels, resulting in a more compact and less permeable morphology (Yuan et al., 2024).

At 10% HA, the morphology exhibits fewer pores than the control but more pores than the 5% HA hydrogel, with moderate interconnectivity. This slight increase in porosity compared to the 5% HA hydrogel may result from the aggregation of HA particles at higher concentrations. The aggregates can create localized regions where pores are formed around the clusters of particles. However, the overall porosity and interconnectivity remain lower than the control due to the higher filler content. The moderate interconnectivity reflects a balance between the polymer-HA interactions and the potential pore-forming effects of particle aggregation.

These findings suggest that increasing hydroxyapatite (HA) content significantly influences the microstructure of the hydrogel, affecting its porosity and interconnectivity. The addition of HA enhances the hydrogel's mechanical strength and bioactivity, making it more suitable for bone regeneration applications. However, an excessive HA concentration may lead to structural changes that could compromise the scaffold's ability to support cell infiltration, nutrient diffusion, and vascularization. For effective bone regeneration, maintaining an optimal balance between mechanical integrity and morphological properties is crucial. Sufficient porosity and nterconnectivity are essential to facilitate cell attachment, proliferation, and extracellular matrix deposition, ultimately supporting new bone tissue formation. Therefore, careful optimization of HA content is necessary to ensure that the hydrogel retains both its enhanced mechanical properties and the structural characteristics required for successful tissue integration and functional regeneration

Elemental Composition Identification

The elemental composition of the hydrogels, analysed using Energy Dispersive X-ray Spectroscopy (EDX), demonstrates significant changes with the addition of hydroxyapatite (HA), reflecting the role of HA and the crosslinkers which are glutaraldehyde and calcium chloride in altering the hydrogel structure. As displays in Table 1, the percentage of each element composition in each hydrogel samples are recorded. For the PVA/Pectin hydrogel, the primary elements detected were oxygen (66.432%), chlorine (20.721%), and calcium (12.848%), with phosphorus being undetectable. The high oxygen content is attributed to the hydroxyl and carboxyl groups present in PVA and pectin, as well as water retained in the hydrogel structure. The significant chlorine concentration arises from calcium chloride, used as a crosslinker, while calcium originates from the same source. The absence of phosphorus indicates that no inorganic phosphate-based components, such as HA, were incorporated into this composition. When 5% HA was added, the elemental composition changed significantly, with oxygen reducing to 47.880%, chlorine decreasing to 16.411%, calcium increasing to 20.305%, and phosphorus appearing at 2.801%. The reduction in oxygen content reflects the partial substitution of the organic polymer matrix with the inorganic HA, which has a lower oxygen ratio compared to the polymer. The increase in

Table 1 Percentage of elements composition

Samples	Elements			
	O (%)	Cl (%)	Ca (%)	P (%)
PVA/Pectin	66.432	20.721	12.848	0
PVA/Pectin/5%HA	47.880	16.411	20.305	2.801
PVA/Pectin/10%HA	50.952	14.941	31.044	3.063

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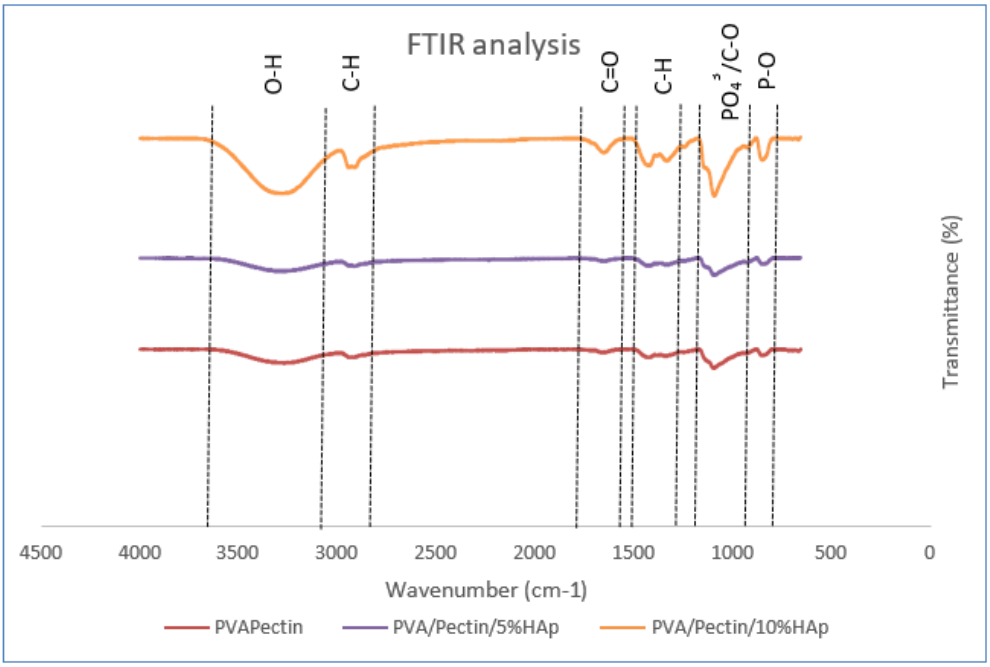

Fig 3 FTIR analysis of the PVA/Pectin hydrogel, PVA/Pectin hydrogel with 5% hydroxyapatite and PVA/Pectin hydrogel with 10% hydroxyapatite

calcium and the appearance of phosphorus confirm the incorporation of HA, which consists of calcium phosphate. The slight decrease in chlorine indicates that the relative contribution of calcium chloride to the composition is reduced due to the presence of calcium from HA. The presence of phosphorus further supports the successful introduction of HA, which enhances the bioactive properties of the hydrogel.

For the hydrogel with 10% HA, oxygen increased slightly to 50.952%, chlorine further decreased to 14.941%, calcium significantly increased to 31.044%, and phosphorus increased to 3.063%. The higher calcium and phosphorus content is directly proportional to the increased HA concentration, as HA is a major source of these elements. The slight increase in oxygen may be attributed to the additional hydroxyl groups present in HA particles or due to changes in the polymer matrix caused by increased filler content. The further reduction in chlorine suggests that the role of calcium chloride as a crosslinker becomes less dominant as the HA particles contribute more calcium to the hydrogel.

These results demonstrate that the incorporation of HA alters the elemental composition of the hydrogel, with higher HA content leading to an increased presence of calcium and phosphorus while reducing the relative contributions of chlorine and organic components. This shift in composition indicates successful incorporation of HA, which is essential for improving the bioactivity and mechanical properties of the hydrogel for bone regeneration applications.

In the PVA/pectin hydrogel with 10% HA, the elemental composition further shifts. Oxygen content slightly increases to 50%, likely due to the hydroxyl groups in HA contributing to the total oxygen. Calcium content increases significantly to 31%, reflecting the higher concentration of HA, while phosphorus also rises to 3%, maintaining the expected Ca/P ratio characteristic of hydroxyapatite. Chlorine content decreases further to 14%, indicating its dilution as the hydrogel becomes increasingly dominated by HA particles. These results demonstrate that increasing HA content directly influences the elemental composition of the hydrogel. The proportional increase in calcium and phosphorus validates the successful incorporation of HA, while the changes in oxygen and chlorine reflect the redistribution of elements due to the interaction between the polymeric and inorganic phases. This elemental analysis confirms the structural and compositional changes within the hydrogel, which are crucial for tailoring its properties for applications like bone regeneration, where calcium and phosphorus play essential roles in promoting bioactivity and osteointegration.

Confirmation of Functional Group Formed in Hydrogel Samples

Fourier Transform Infrared Spectroscopy (FTIR) analysis confirms the presence of functional groups in the hydrogel samples and reveals how the incorporation of hydroxyapatite (HA) influences the chemical interactions within the matrix. The observed peaks in the FTIR spectra correspond to characteristic functional groups of the PVA/pectin hydrogel and the added HA. For the PVA/pectin hydrogel, the broad peak at 3277.67 cm^{-1} corresponds to O-H stretching vibrations, indicative of the hydroxyl groups from PVA and pectin, as well as water molecules retained in the hydrogel. The peak at 2940.02 cm^{-1} represents-H stretching vibrations from the polymer backbone, while the prominent peak at 1647.05 cm^{-1} is attributed to C=O stretching, associated with ester and aldehyde groups from pectin and the glutaraldehyde crosslinker. The peak at 1418.30 cm^{-1} corresponds to C-H bending, and the peaks at 1088.70 cm^{-1} and 841.78 cm^{-1} are attributed to PO_4^{3-} groups and C-O stretching, reflecting the functional groups of pectin and crosslinking interactions (Kim et al 2005; Mansur et al., 2008).

In the hydrogel with 5% HA, the O-H peak shifts slightly

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to 3286.08 cm^{-1}, suggesting enhanced hydrogen bonding due to the interaction of the polymer matrix with HA particles. The C-H stretching peak shifts to 2913.73 cm^{-1}, and the C=O peak slightly shifts to 1645.24 cm^{-1}, reflecting changes in the polymer matrix caused by the presence of HA. These shifts indicate alterations in the chemical environment and possible interactions between HA and functional groups of the polymer, such as hydroxyl and carboxyl groups. The PO_4^{3-} peak at 1090.25 cm^{-1} is more pronounced, confirming the incorporation of phosphate groups from HA. Similarly, the P-O peak at 847.61 cm^{-1} becomes stronger, further confirming the presence of HA within the hydrogel (Kim et al 2005; Mansur et al., 2008).

For the hydrogel with 10% HA, the O-H stretching peak shifts to 3267.03 cm^{-1}, indicating further changes in hydrogen bonding due to the increased HA content. The C-H peak at 2936.14 cm^{-1} and the C=O peak at 1649.78 cm^{-1} also exhibit slight shifts, suggesting stronger interactions between the HA and the polymer matrix. The PO_4^{3-} peak at 1091.90 cm^{-1} and the P-O peak at 845.82 cm^{-1} become even more prominent, reflecting the higher concentration of HA. These results indicate that the HA interacts with the polymer network, altering the chemical environment and functional group behaviour (Kim et al., 2005; Mansur et al., 2008).

Overall, the FTIR results confirm the presence of characteristic functional groups of PVA, pectin, and HA. The shifts in peak positions and intensities with increasing HA content demonstrate strong interactions between the polymer matrix and HA particles, which are critical for enhancing the hydrogel's bioactivity and mechanical properties for bone regeneration applications. The crosslinking agents, glutaraldehyde and calcium chloride, contribute to the hydrogel structure by stabilizing the polymer network and promoting the integration of HA matrix.

CONCLUSION

In conclusion, the hybrid hydrogel composed of PVA/pectin and incorporated with hydroxyapatite (HA) demonstrates significant potential for bone regeneration applications, with 5% HA identified as the optimal formulation based on the results and analyses. The gelling time of the hydrogel increases with HA content, with the 5% HA hydrogel showing a reasonable balance of gelling time which is 1.5 hours was practical for clinical applications. Surface morphology analysis via SEM reveals that the 5% HA hydrogel achieves a balance between porosity and interconnectivity. While it has slightly reduced porosity and diminished interconnectivity compared to the control, it maintains sufficient pore structure for cell infiltration and tissue integration. Therefore, the PVA/pectin hydrogel with 5% HA is the most favourable formulation, offering excellent bioactivity, manageable injectability, and suitable structural characteristics for bone regeneration applications. It represents a promising candidate for further development and clinical implementation in tissue engineering.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICS DECLARATION

This study did not involve human participants, animal subjects, or sensitive data. Consequently, formal ethical approval was not required

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