

Original Research Article

Next-generation hybrid bovine pericardium–hyaluronic acid membranes with biofunctional properties: FTIR, XRD and SEM characterization

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Abstract

Purpose: To evaluate bovine pericardium membranes modified with 120 kDa hyaluronic acid (HA) for their physicochemical and biofunctional properties.

Methods: Bovine pericardium membranes were soaked in different concentrations of HA solutions (0.5, 1.0 and 2.0 %) and analyzed using Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups, X-ray Diffraction (XRD) to assess crystallinity, and Scanning Electron Microscopy (SEM) combined with Energy-Dispersive X-ray Spectroscopy (EDS) was used to examine surface morphology and carry out elemental composition analysis.

Results: The FTIR analysis revealed a stable collagen structure with preserved amide peaks and hydroxyl groups, indicating biochemical integrity. In contrast, the X-ray diffraction (XRD) results showed that all groups exhibited amorphous structures, suggesting flexibility suitable for soft tissue regeneration. Scanning electron microscopy showed a uniform morphology, while elemental analysis indicated that carbon (C), nitrogen (N), and oxygen (O) were the major elements present, with oxygen content increasing at higher HA concentrations, reflecting improved hydrophilic properties.

Conclusion: Bovine pericardium membranes modified with 120 kDa HA exhibit preserved structural integrity, increased hydrophilicity, and uniform surface morphology, indicating their overall potential as scaffolds for wound repair and soft tissue engineering. Further optimization is necessary to improve degradation resistance and crystallinity for wider biomedical applications.

Keywords: Bovine pericardium membrane, Hyaluronic acid, FTIR, XRD, SEM

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INTRODUCTION

Biomaterials are increasingly important in regenerative medicine. Bovine pericardium membrane offers high mechanical strength and elasticity, while hyaluronic acid (HA) enhances hydrophilicity and cellular interaction. The use of biocompatible biological materials provides innovative solutions in tissue regeneration, implants, and wound treatment. One of the most widely used biomaterials is bovine pericardium membrane, which exhibits high mechanical strength, elasticity, and compatibility with human tissues. However, to enhance its performance and bioactive properties, bovine pericardium membrane can be combined with bioactive compounds such as hyaluronic acid [1,2].

Hyaluronic acid is a natural polysaccharide with biocompatible and biodegradable properties, which can support the tissue regeneration process. Hyaluronic acid is frequently applied in medical treatments, including joint lubrication, wound dressing, and tissue regeneration. Inflammation is a complex process involving pain, membrane changes, protein denaturation, and increased vascular permeability [3]. Hyaluronic acid with a low molecular weight, such as 120 kDa, retains moisture, reduces inflammation, and accelerates wound healing.

Characterization of biomaterials is essential to understand their physicochemical and morphological properties. Fourier Transform Infrared Spectroscopy (FTIR) is used to identify functional groups and chemical interactions between material components. X-Ray Diffraction (XRD) helps identify the properties of crystallinity and changes in the structure of materials due to the modification process. Furthermore, the Scanning Electron Microscope (SEM) provides a detailed overview of surface morphology and component distribution in biomaterials [4].

Bovine pericardium membrane modified with hyaluronic acid is anticipated to exhibit optimal porosity, support cell proliferation, and maintain stable mechanical properties. Furthermore, these membranes are expected to demonstrate enhanced resistance to enzymatic degradation, rendering them suitable for long-term biomedical applications. This study aims to comprehensively characterize these properties using FTIR, XRD, and SEM [5]. The findings of this study are expected to contribute to the development of innovative and functional biomaterials, providing insights into the optimization of natural membrane-based materials and bioactive compounds for broader applications in tissue and implant regeneration [6].

EXPERIMENTAL

Design

This study employed a quantitative approach to develop and characterize bovine pericardium membranes modified with 120 kDa hyaluronic acid. It focused on analyzing the physicochemical properties and morphological characteristics of the resulting biomaterials using instrumental characterization techniques [7].

Sample collection and preparation

The sample used in this study consisted of bovine pericardium membranes (5 × 5 cm) obtained from slaughterhouses that complied with hygiene and research ethics standards. Each membrane was cleaned by rinsing with sterile saline solution to remove residual tissue and blood, followed by dehydration and sterilization prior to characterization. The membranes were then immersed in 120 kDa hyaluronic acid (HA) solutions at concentrations of 0.5, 1.0 and 2.0 % for 24 h to achieve surface modification [8].

Sample analysis

Properties of the treated sample were analyzed using three main characterization techniques.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was performed using a [brand/model] spectrometer in the range of 4000 – 400 cm⁻¹, with a resolution of 4 cm⁻¹ and 32 scans per sample, using an ATR diamond crystal. This setup was used to identify functional groups and chemical interactions between the bovine pericardium membrane and hyaluronic acid.

X-Ray Diffraction (XRD)

X-Ray Diffraction (XRD) analysis was conducted using a diffractometer with Cu K α radiation (λ = 1.5406 Å), operated at 40 kV and 30 mA. Data were collected over a 2 θ range of 5 – 60° at a scanning rate of 2°/min. This method was employed to determine structural changes in membrane crystallinity resulting from modification [14].

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was performed using a [brand/model] microscope at

Taken together, these spectral characteristics demonstrate that hyaluronic-acid modification does not disrupt the principal chemical signatures of the bovine pericardium membrane. The preservation of hydroxyl, aliphatic, amide, and C–O functional groups indicates maintained structural integrity and suggests that the modified membranes retain favorable biocompatibility, supporting their potential use in biomedical applications.

X-Ray Diffraction (XRD) analysis

The XRD evaluation of bovine pericardium membranes modified with 0.5%, 1.0%, and 2.0% 120 kDa hyaluronic acid (Figure 2) revealed a consistent amorphous diffraction pattern, characterized by a broad halo within the 2θ range of 20° – 30° . No measurable crystalline reflections were observed at any concentration, indicating that the structural arrangement of the membranes remained predominantly non-crystalline and was not affected by variations in hyaluronic acid content.

The absence of distinct diffraction peaks in all samples prevented the determination of crystallite size using standard XRD-based calculations. This uniform amorphous behavior reflects the intrinsic non-crystalline characteristics of both the bovine pericardium matrix and hyaluronic acid, as well as the lack of mineralized constituents capable of forming ordered lattice structures. As a result, the degree of crystallinity remained consistently low, suggesting that the development of organized crystalline phases would require the addition of mineral components with defined crystal-forming capacity.

When assessed against reference criteria for hard tissue biomaterials, typically require crystallite sizes in the range of 20 – 100 nm and crystallinity values of 50 – 70 %. The samples did not meet these established benchmarks. The predominance of amorphous features across all formulations confirms the absence of significant crystalline phases, indicating that the current membrane–hyaluronic acid combinations are unsuitable for high-crystallinity applications unless further mineral reinforcement is introduced.

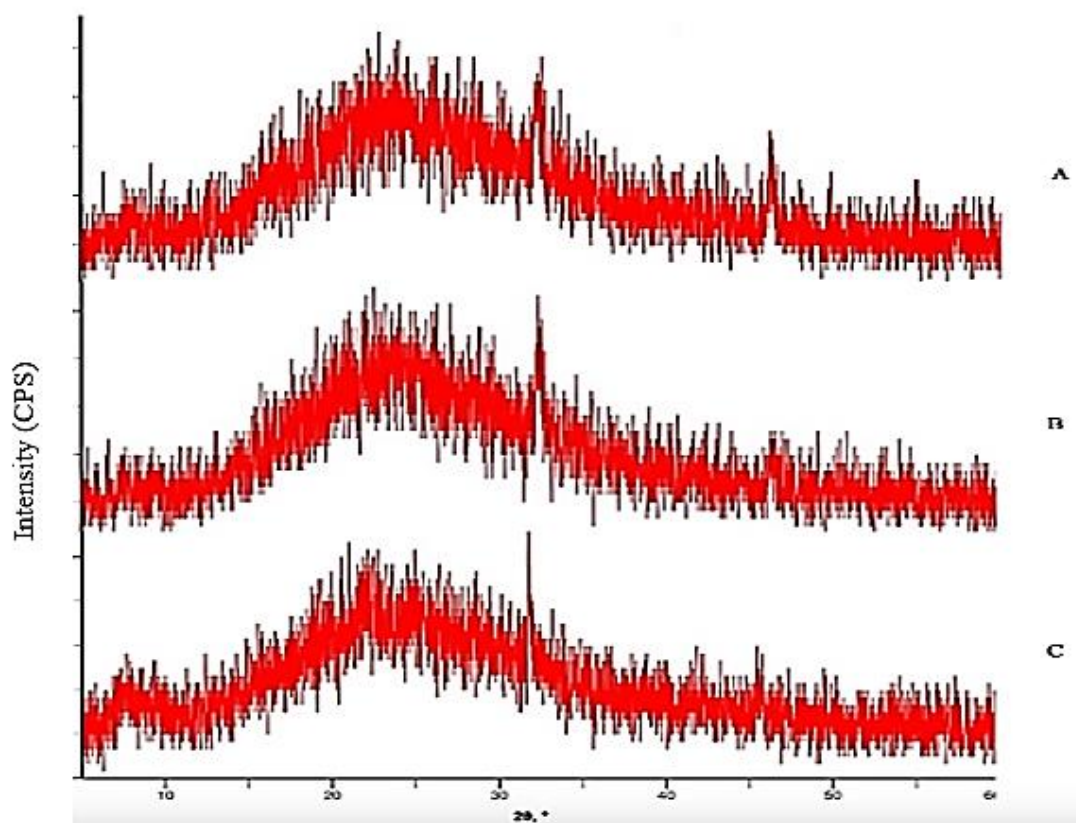


Figure 2: XRD analysis of bovine pericardium membrane modified with (A) 0.5 %, (B) 1.0 %, (C) 2.0 % 120 KDa hyaluronic acid

Scanning electron microscope (SEM) analysis

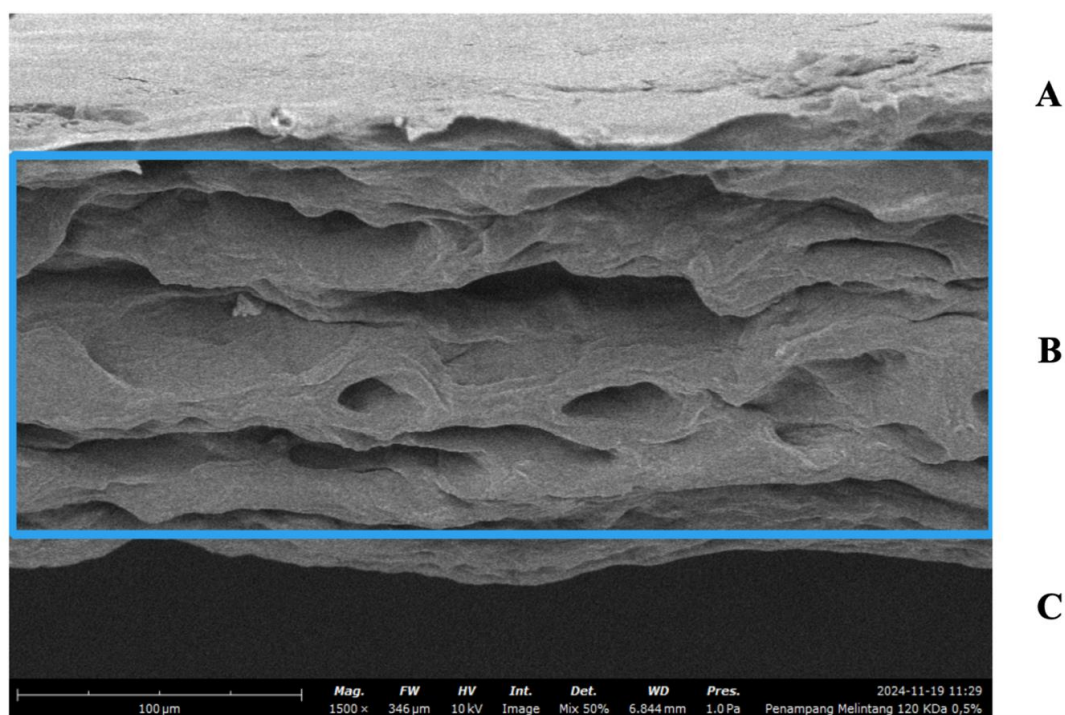


Figure 3: Scanning electron micrograph (SEM) cross-sectional micrograph of the bovine pericardium membrane modified with 0.5 % 120 KDa hyaluronic acid, showing three distinct structural regions: (a) the outer surface layer, (b) the middle lamellar collagen layer, and (c) the inner compact layer. image acquired at 10 KV in mixed detector mode. Scale bar: 100 μ m

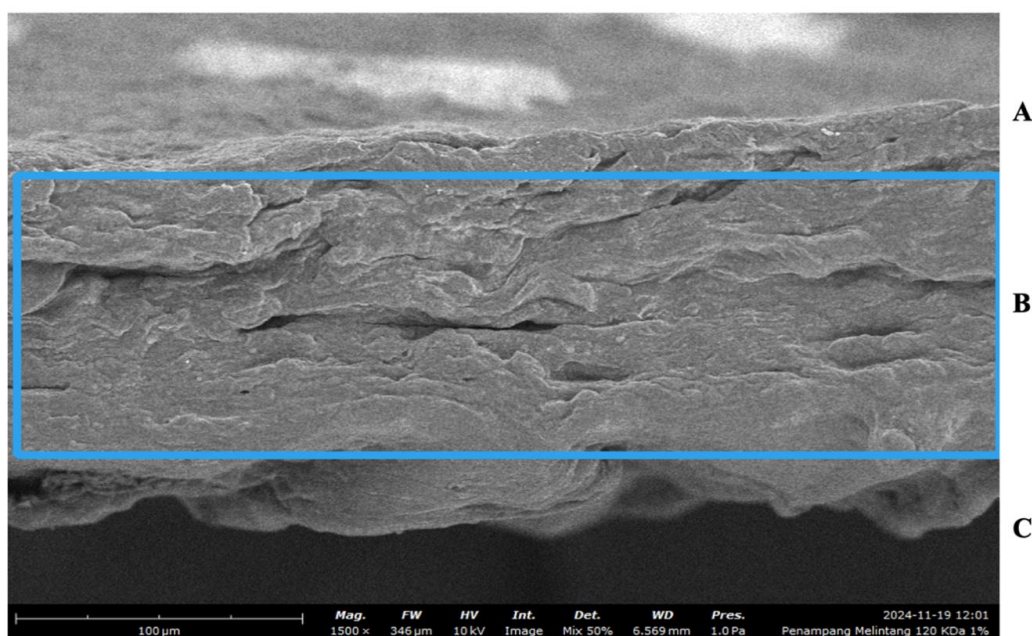


Figure 4: Cross-sectional SEM micrograph of the bovine pericardium membrane modified with 1.0% 120 kDa hyaluronic acid. The image was captured at 10 kV with a 346 μ m field width using a 50% mixed detector mode. Three structurally distinct regions of the membrane are clearly visible and have been labeled in the micrograph: (A) the outer dense surface layer, (B) the middle collagenous fibrous layer, and (C) the inner loose layer. These regions reflect the natural hierarchical architecture of the bovine pericardium following hyaluronic-acid modification

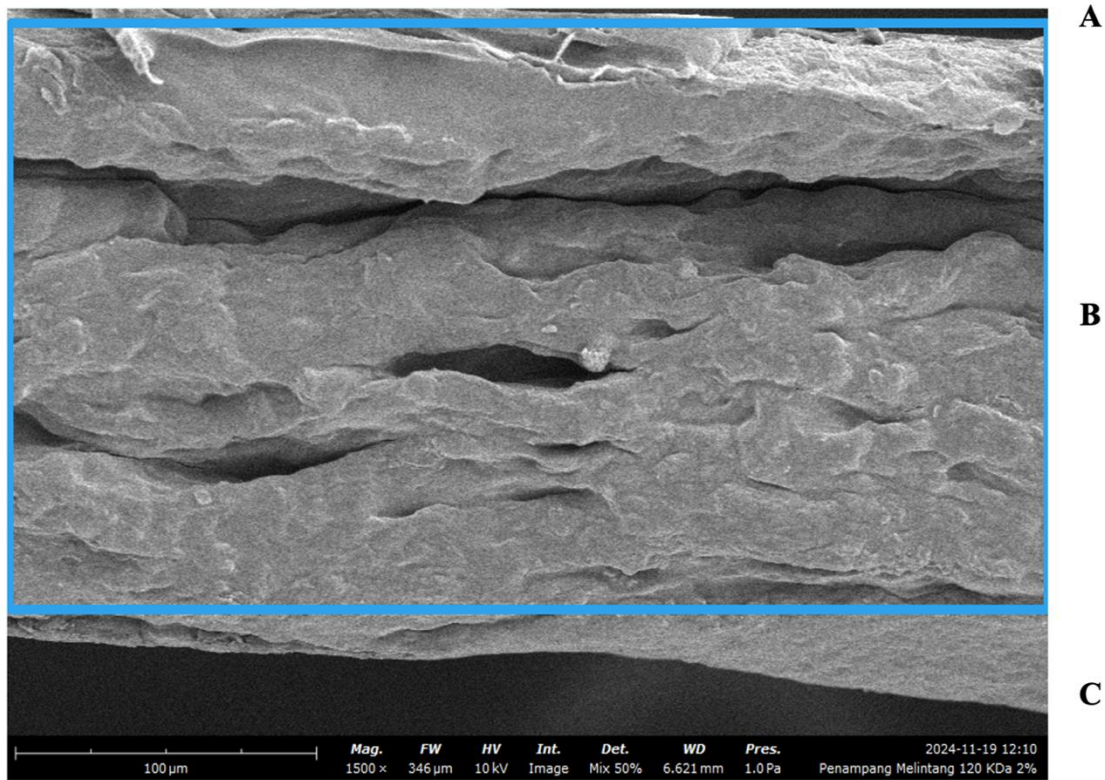


Figure 5: Cross-sectional SEM micrograph of the bovine pericardium membrane modified with 2.0 % 120 KDa hyaluronic acid. The SEM image was captured at 10 KV accelerating voltage with a 346 μ m field width and 50% mixed detector mode. three distinct structural regions have been identified and labeled in the micrograph: (A) the outer dense surface layer, (B) the middle collagenous fibrous layer, and (C) the inner loose layer. These regions collectively represent the characteristic hierarchical architecture of the bovine pericardium following modification with hyaluronic acid

Table 1: SEM–EDS elemental composition of the bovine pericardium membrane modified with 0.5 % 120 kDa Hyaluronic acid, showing the atomic and weight percentages of carbon (C), nitrogen (N), and oxygen (O)

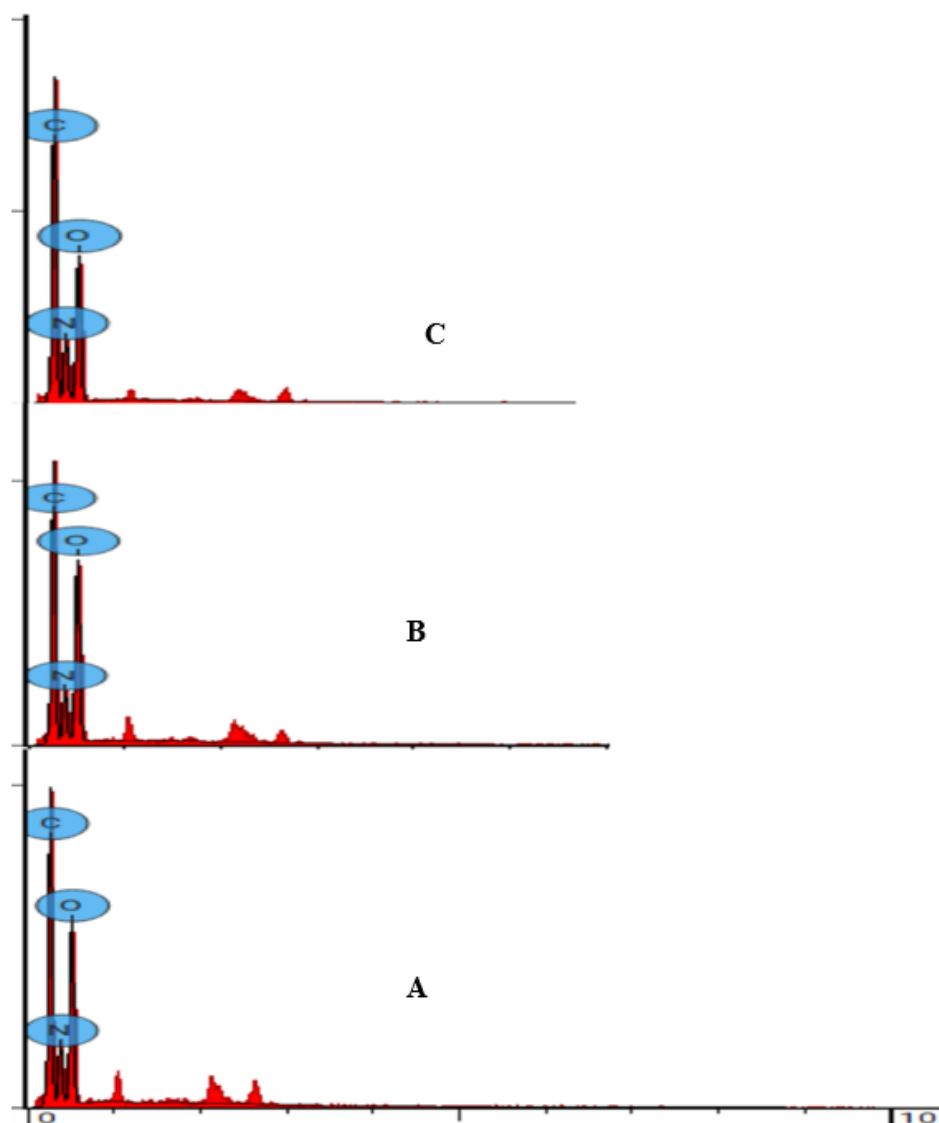
Element number	Element symbol	Element name	Atomic concentration (% at)	Weight concentration (% wt)
6	C	Carbon	36.331	31.500
7	N	Nitrogen	35.099	35.500
8	O	Oxygen	28.570	33.000

Table 2: SEM–EDS elemental composition of the bovine pericardium membrane modified with 1.0 % 120 kDa Hyaluronic acid, showing the atomic and weight percentages of carbon (C), nitrogen (N), and oxygen (O)

Element number	Element symbol	Element name	Atomic concentration (% at)	Weight concentration (% wt)
6	C	Carbon	34.672	29.800
7	N	Nitrogen	32.316	32.400
8	O	Oxygen	33.012	37.800

Table 3: SEM–EDS elemental composition of the bovine pericardium membrane modified with 2.0 % 120 KDa hyaluronic acid, showing the atomic and weight concentrations of carbon (C), nitrogen (N), AND oxygen (O)

Element number	Element symbol	Element name	Atomic concentration (% at)	Weight concentration (% wt)
6	C	Carbon	34.980	30.000
7	N	Nitrogen	30.186	30.200
8	O	Oxygen	34.834	39.800



Figures 6: Scanning Electron Microscopy (SEM) micrographs and corresponding Energy Dispersive X-ray Spectroscopy (EDS) spectra of bovine pericardium membranes modified with 0.5, 1.0 and 2.0 % 120 kDa Hyaluronic Acid, respectively

The SEM images depict the surface morphology of each sample, while the EDS spectra reveal the predominant elemental composition, primarily carbon (C), oxygen (O), and nitrogen (N). Data acquisition was performed over 30 seconds, yielding total counts of 22,796 (755 counts/s), 18,462 (611 counts/s), and 19,302 (639 counts/s) for the respective samples. To avoid spectral interference and ensure accurate identification of elemental peaks, elements such as Au, Ti, Na, Nb, Zn, Cl, and B were excluded during the analysis.

The elemental composition of bovine pericardium membranes modified with 0.5, 1.0, and 2.0 % 120 kDa hyaluronic acid (Figure 3 to Figure 6) was analyzed using SEM–EDX. The results consistently indicated that carbon (C),

nitrogen (N), and oxygen (O) were the primary elements, reflecting the organic nature of the membranes and the presence of collagen, proteinaceous components, and hydroxyl or carboxyl groups from hyaluronic acid.

For the 0.5 % HA-modified membrane, the atomic concentrations of C, N, and O were 36.33, 35.10, and 28.57%, respectively, with weight concentrations of 31.5, 35.5 and 33.0 %. The 1.0 % HA-modified sample exhibited slightly lower C (34.67 %) and N (32.32 %) levels, whereas O increased to 33.01 % (weight: 37.8 %). The 2.0 % HA sample showed further increase in O (34.83 %, weight: 39.8 %), with minor decreases in C (34.98 %) and N (30.19 %, weight: 30.2 %).

The SEM – EDX measurements presented in Table 1 to Table 3 were performed under consistent operational conditions, including an accelerating voltage of 10 kV, a field width of 346 μm , mixed detector mode (50 %), and acquisition times recorded for each sample. Elements such as Au, Ti, Na, Nb, Zn, Cl, and B were excluded to prevent interference with elemental identification. Overall, the data confirm that the membranes are predominantly composed of organic elements. Oxygen content increased proportionally with HA concentration, suggesting enhanced hydrophilicity and potential for improved cell adhesion and wound healing.

DISCUSSION

The FTIR analysis demonstrated that the characteristic structural features of key extracellular matrix proteins, particularly collagen, which is the predominant structural component of the bovine pericardium, were retained following the modification process. The preservation of these molecular structures is essential because collagen plays a pivotal role in maintaining the mechanical integrity and biofunctional performance of the membrane [11]. The detected amide clusters indicate that the material possesses functional groups capable of interacting with components of the extracellular matrix (ECM), thereby enhancing biological integration. Potential applications include wound dressings, scaffolds for soft tissue regeneration, and implants that require high biocompatibility [12].

However, XRD characterization revealed that this material exhibited an amorphous pattern at all tested hyaluronic acid concentrations (0.5 – 2.0 %). This amorphous property is ideal for soft tissue applications due to its flexibility, but it does not provide sufficient mechanical support for hard tissues such as bones or teeth [13]. Unless there is an addition of mineralization (such as hyaluronic acid), this material will retain its amorphous character without a clear crystalline peak. In biomaterials for hard tissue applications, the size of the crystallite for crystalline components typically ranges from 20 – 100 nm, with a degree of crystallinity of 50 – 70 %, depending on the application. The degree of crystallinity will only increase if crystalline components or minerals are added. Low crystallinity indicates that this material remains amorphous with a low degree of crystallinity [14]. In the Discussion section, we elaborate that if increased crystallinity is required for specific applications, such as in biomaterials for hard tissues, mineralization with crystalline

components (e.g., hyaluronic acid) can result in sharper diffraction patterns at around $2\theta = 31 - 32^\circ$. Therefore, for applications on hard tissues, combinations with bioactive or structural components such as hyaluronic acid can be considered. Previous research has shown that hyaluronic acid can increase the degree of crystallinity and mechanical strength of biomaterials without compromising their biocompatibility. The main challenge in this modification is to ensure that the mineralization process does not reduce the even distribution of hyaluronic acid on the membrane, as the modification can affect its bioactive properties. A low degree of crystallinity means that this material remains amorphous with a low degree of crystallinity and does not show an increase in crystallinity with the addition of hyaluronic acid to this concentration. If increased crystallinity is required, e.g., for biomaterial applications in hard tissues, mineralization with crystalline components such as hyaluronic acid can result in a more pronounced crystallization pattern at a specific angle (e.g., a typical diffraction peak at $2\theta = 31 - 32^\circ$ for hyaluronic acid).

Scanning Electron Microscope (SEM) analysis provides additional information regarding the morphology of materials, especially the distribution of key elements such as carbon, nitrogen, and oxygen. The 2.0 % hyaluronic acid with a molecular weight of 120 kDa showed increased oxygen content, suggesting improved oxidative stability and potential bioactivity. These hydrophilic properties may contribute to faster wound healing by creating a moist environment that supports cellular activity. However, the homogeneous distribution of hyaluronic acid should be further studied to ensure there are no areas that are with excessively high or low concentrations of the component, which could affect the overall performance of the material [15].

In addition, a key challenge in developing this biomaterial is its resistance to enzymatic degradation. In medical applications, excessively rapid degradation may compromise the material's functionality. Therefore, further studies are required to elucidate the mechanisms of *in vitro* and *in vivo* degradation and to assess the influence of specific biological factors, including enzymatic activity and mechanical stress, on membrane structure. The addition of crosslinking agents may serve as a strategy to control the rate of degradation without compromising the bioactive properties of materials [16,17].

A biomaterial based on bovine pericardium membrane combined with hyaluronic acid has

great potential for applications in soft tissue regeneration, particularly in wound dressings and biological scaffolds. However, to increase the flexibility of its use, further optimization is needed regarding crystallinity, enzymatic degradation, and distribution of its components. Overcoming these challenges requires a multidisciplinary strategy that combines advanced characterization techniques with rigorous biological evaluation. This integrated approach is expected to enhance the potential of these biomaterials for meaningful contributions in medical applications [18].

CONCLUSION

Biomaterials based on bovine pericardium membranes modified with 120 kDa 2.0 % hyaluronic acid of 120 kDa demonstrate promising potential for soft tissue regeneration, particularly in wound dressings and scaffold applications. The material preserves key protein structures, exhibits favorable hydrophilic and amorphous properties, and supports cellular proliferation and migration. Further studies are needed to optimize hyaluronic acid distribution, control enzymatic degradation, and tailor mechanical properties for broader clinical applications.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Ethical approval

None provided.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dwi Wahyu Indrawati was responsible for the conceptualization and methodology along with Ernie Maduratna Setiawatie, as well as formal analysis together with Retno Pudji Rahayu. The investigation was conducted by Rini Devijanti

Ridwan and Hendrik Setia Budi, while data curation was carried out by Coen Pramono, Ida Bagus Narmada, and Anita Yulianti. Dwi Wahyu Indrawati prepared the original draft of the manuscript, and the review and editing were performed by Devi Rianti, Dwi Wahyu Indrawati, and Rizky Brilliant Syah Manurung. Visualization was handled by RBSM, and overall supervision was provided by Hari Basuki Notobroto. All authors have read and approved the final version of the manuscript.

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