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ORIGINAL ARTICLE

Bovine Amnion–Alginate Sponge (BAAS) as a Modern Wound Dressing: Pilot Study of Functional Groups and BAAS Formulation Effects on Basic Fibroblast Growth Factor and Interleukin-6 Levels

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Abstract

Background/Aim: Chronic wounds are a significant health burden, impacting millions globally and incurring high healthcare costs. Bovine amnion membrane (BAM) offers anti-inflammatory, antimicrobial properties and bioactive growth factors, making it a promising candidate for wound healing applications. At the same time, alginate enhances the stabilisation and delivery of growth factors. This study aimed to characterise a bovine–amnion–alginate sponge (BAAS) by analysing its functional groups and evaluating the interleukin-6 (IL-6) and basic fibroblast growth factor (bFGF) levels in different ratios.

Methods: A laboratory-based experimental study with a randomised, post-test-only design was conducted. BAAS at ratios of 25 %: 75 %, 50 %: 50 % and 75 %: 25 % were crosslinked with $CaCl_2$. The Fourier transform infrared spectroscopy (FTIR) was used to assess functional groups and provide insights into the molecular composition of the samples. Meanwhile, the biomolecules of IL-6 and bFGF levels were quantified using enzyme-linked immunosorbent assay (ELISA).

Results: Chemical functional group analysis using FTIR confirmed ionic bonding between Ca^{2+} ions and carboxylate groups, glycosidic stability and enhanced hydrophilicity in BAM-rich formulations. IL-6 levels were 45, 49 and 56 pg/mL, while bFGF levels were 42, 46 and 52 pg/mL for BAM-alginate ratios of 25 %: 75 %, 50 %: 50 % and 75 %: 25 %, respectively. No IL-6 or bFGF was detected in the pure alginate control.

Conclusion: BAAS formulations demonstrate the interplay between functional groups identified in FTIR spectra and cytokine/growth factor modulation observed in ELISA as evidence that its composition can be tailored to address specific phases of wound healing.

Key words: Amnion; Cattle; Alginates; Sponge; Biomolecules; Wounds and injuries; Chronic.

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Introduction

Chronic wounds pose a global health challenge, requiring innovative approaches to accelerate healing and prevent complications. Current therapeutic strategies have shown varying success, including advanced dressings and bioengineered skin substitutes. Still, a critical need remains for

more effective and accessible biomaterials to enhance wound healing outcomes.[1, 2]

Bovine amnion (BA) membrane has been identified as a potential biomaterial due to its anti-inflammatory and antimicrobial properties and its content of growth factors that support epithelialisation and tissue regeneration. Bovine amnion membrane (BAM) offers advantages over human amniotic membranes, including greater availability, optimal thickness and the ability to reduce scar tissue formation.[3, 4] Among the critical growth factors in wound healing, the essential fibroblast growth factor (bFGF) is pivotal in proliferative by stimulating fibroblast proliferation, angiogenesis and extracellular matrix deposition.[5, 6] Meanwhile, interleukin 6 (IL-6), in particular, is a key mediator in shifting the wound microenvironment from an inflammatory state to active regeneration, highlighting its relevance in the therapeutic application of BAMs.[7]

Alginate, a naturally occurring polysaccharide derived from brown algae, possesses unique properties, making it an ideal adjunct in wound healing. Using alginate as a supporting component in combination with BAM further enhances the effectiveness of this biomaterial. Alginate can absorb wound exudate, maintain moisture and modulate cytokine activity, such as IL-6, which plays a role in transitioning from the inflammatory phase to the proliferative phase.[8]

The bovine amnion-alginate combination is expected to provide an effective and economical solution for managing chronic wounds. Despite its promising properties, the clinical application of BAM-alginate requires careful consideration of processing techniques, including decontamination steps such as antibiotic and antimycotic treatment, to ensure sterility. Furthermore, rigorous characterisation of BAM's biocompatibility and immunogenicity is essential to minimise adverse reactions in human applications.

Methods

Study design

This experimental study used a post-test-only group design to analyse the characteristics of bovine amnion–alginate sponge (BAAS) in various concentrations. The samples were processed and prepared by the Cell and Tissue Bank-Regenerative Medicine Centre of Dr Soetomo General Academic Hospital in Surabaya, East Java, Indonesia. The sample was divided into several groups, including alginate sponge (Control), BA + alginate sponge (25 %: 75 %), BA + alginate sponge (50 %: 50 %) and BA + alginate sponge (75 %: 25 %). This study aimed to evaluate the potential of the BAM-alginate combination as a modern wound dressing. The biomaterial characteristics were analysed using Fourier transform infrared spectroscopy (FTIR) to assess its chemical functional group interactions and enzyme-linked immunosorbent assay (ELISA) measurements of IL-6 and bFGF levels as *in vitro* indicators of their biological properties supporting the wound healing process.

Preparation of BAAS

Preparing a BAAS is illustrated in Figure 1. The process began with sourcing and processing its key components: BAM and alginate. First, the BAM was saturated in NaCl 0.05 % solution for 10 min. The amnion membrane was then re-washed with distilled water until clean from the NaCl solution. The BAM chorion was separated, cleaned from blood clots and washed with distilled water. The membrane was treated with an antibiotic-antimycotic solution (penicillin G 200 IU/mL, streptomycin 200 µg/mL and amphotericin B 2.5 µg/mL) for 30 minutes to ensure sterility. In the next step, the cleaned BAM was stretched on a sheet covered with gauze. The BAM was put in the freezer at –80 °C for 1 × 24 h, then freeze-dried for 1 × 24 h at –100 °C to make a dried BAM. [9]

The sponge combined a ground amniotic membrane with alginate and calcium chloride as cross-linking agents in the desired proportion. The mixture was moulded as sponge preparation and stored in a deep freezer at -80 °C for at least 24 h to make a freeze-dried amnion-alginate sponge. The total number of samples for each group was 6. The final sponge thickness was 5 mm, a critical factor influencing mechanical properties and degradation rates in wound healing applications.

FTIR analysis

This method allows for identifying functional groups and provides insights into the molecular composition of the samples. The sample was mixed with potassium bromide (KBr) in a ratio of 1:4. The mixture was ground until homogeneous

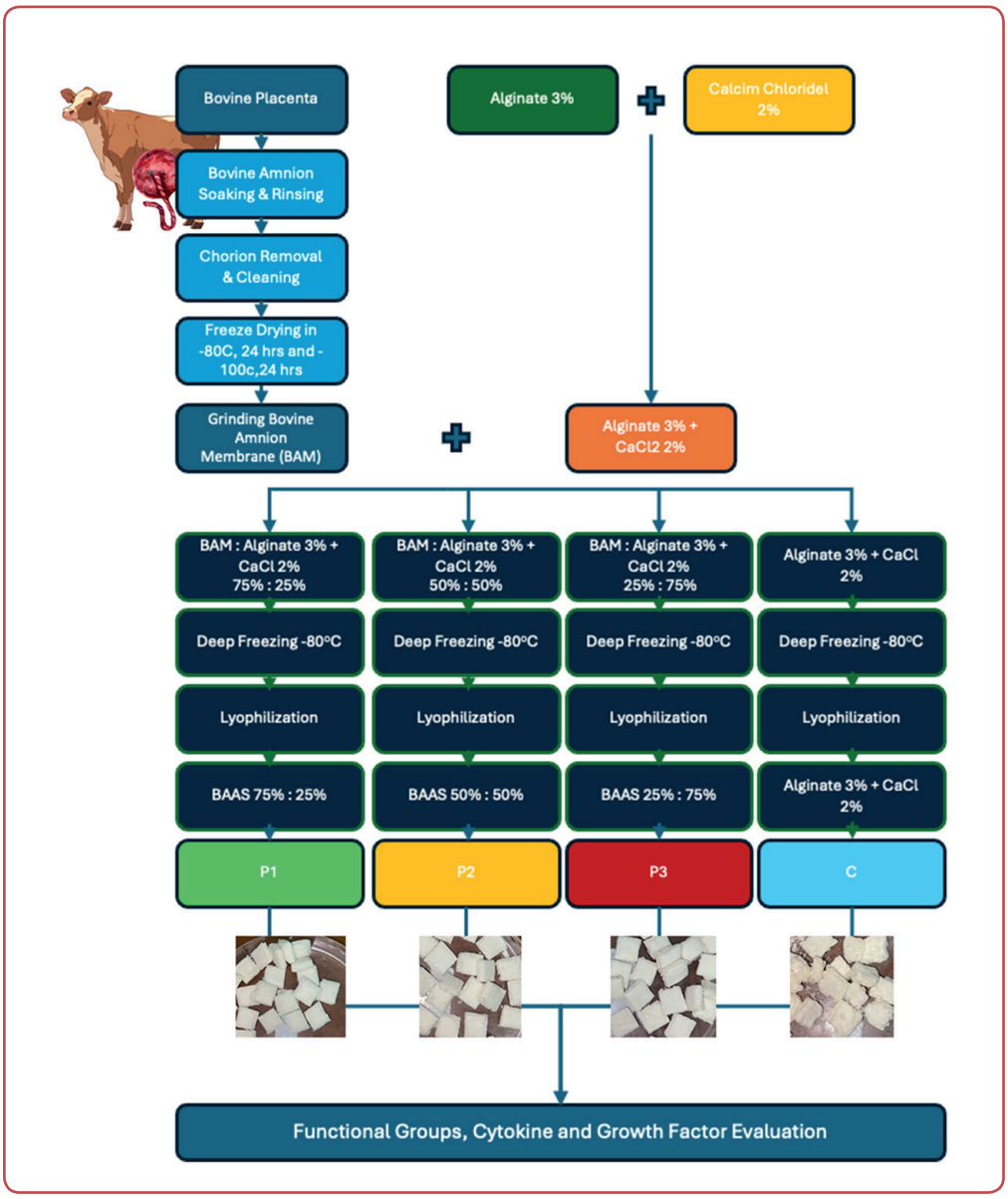


Figure 1: Schematic flowchart for bovine amnion–alginate sponge (BAAS) preparation and evaluation

and placed in a pan with a diameter of 4 mm. The analysis used IR Prestige-21 (Shimadzu, Japan) in 400–4000 cm^{-1} and 2.0 cm^{-1} resolutions and the value will appear as transmittance percentage.

Testing the cytokine and growth factor levels

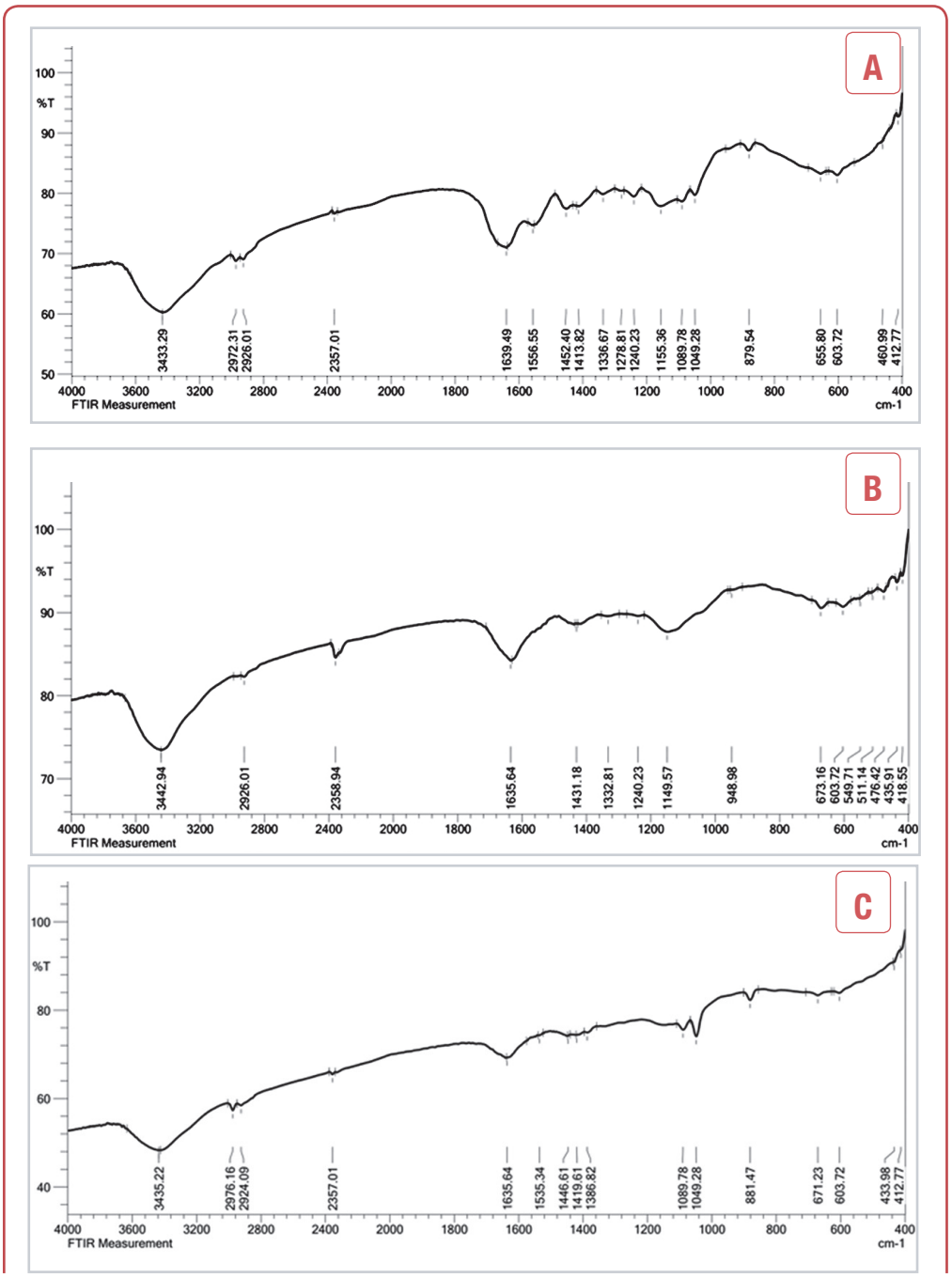
The sample weighing 20 mg of BAAS and control was collected and pulverised. The pulverised sample was then dissolved in 1 cc of 0.9 % NaCl. The sonicator water bath (ELMA 1040 H) was used to homogenise the mixture for 10 minutes, followed by centrifugation at 1500 rpm for 5 minutes. The supernatant was then collected to analyse the levels of IL-6 and bFGF using an ELISA kit (RayBio Bovine IL-6 and bFGF ELISA kits). The detection limits for IL-6 and bFGF were 3.9 pg/mL and 15 pg/mL, respectively. The absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA) and the cytokine and growth factor concentrations were calculated based on standard curves generated from known concentrations.[6]

Data analysis

A comparative description of each sample spectra was used to determine the chemical functional group. The cytokine and growth factor data were analysed using the One-way ANOVA test to assess cytokine and growth factor levels. Pairwise analysis of each sample was evaluated using the least significant difference (LSD) test as post hoc analysis.

Results

The FTIR analysis of BA with varying alginate + $CaCl_2$ ratios (25: 75, 50: 50 and 75: 25) revealed distinct trends in functional group interactions (Figure 2). Increased BA content amplified hydroxyl (-OH) peaks (3430–3450 cm^{-1}), indicating enhanced hydrogen bonding and hydrophilicity. Carbonyl (C=O) peaks (1630–1650 cm^{-1}) became more intense with higher BA ratios, suggesting stronger molecular interactions and improved crosslinking, contributing to structural stability. Alkyl (C-H) stretching peaks (2920–2930 cm^{-1}) and broader fingerprint region peaks confirmed the integration of BAM into the alginate matrix.



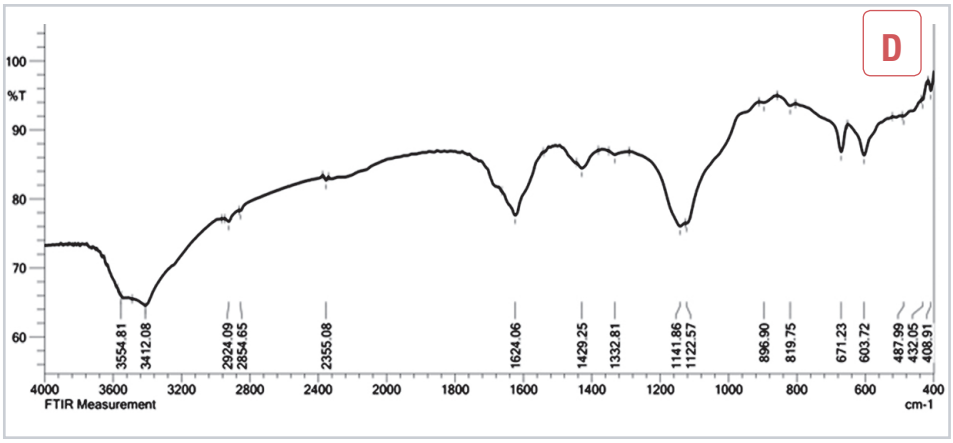


Figure 2: The Fourier transform infrared spectroscopy (FTIR) spectrum: (A) Bovine amnion–alginate sponge (BAAS) (25 %: 75 %); (B) BAAS (50 %: 50 %); (C) BAAS (75 %: 25 %); (D) Alginate (Control);

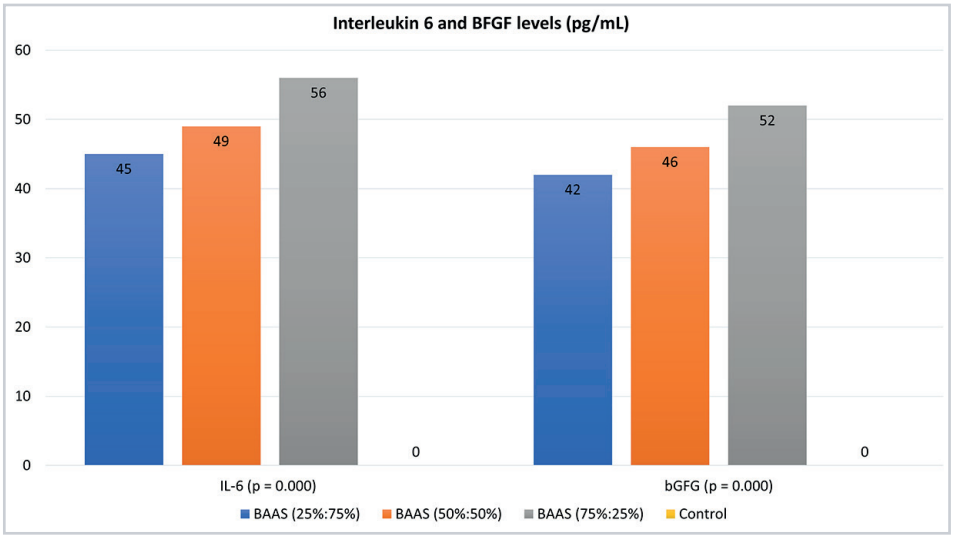


Figure 3: Interleukin 6 and basic fibroblast growth factor (bFGF) levels analysis using ANOVA
BAAS: bovine amnion–alginate sponge;

Table 1: Post hoc analysis of interleukin-6 (IL-6) and basic fibroblast growth factor (bFGF) using LSD test

Parameter	BAAS (25 %: 75 %)	BAAS (50 %: 50 %)	BAAS (75 %: 25 %)	Control
BAAS (25 %: 75 %)		0.000	0.000	0.000
BAAS (50 %: 50 %)	0.000		0.000	0.000
BAAS (75 %: 25 %)	0.000	0.000		0.000
Control	0.000	0.000	0.000	

BAAS: bovine amnion–alginate sponge; Values in tables are p-values of LSD test;

Cytokine and growth factor analysis

The *in vitro* results of IL-6 bFGF levels obtained through ELISA for different formulations provide insight into how varying BA ratios influence cytokine and growth factor levels. The ANOVA test confirmed that differences in IL-6 and bFGF levels between groups were statistically significant ($p = 0.000$; $p = 0.000$), respectively (Figure 3). Pairwise comparisons in the post hoc analysis further supported the different cytokine and growth factor levels on BAAS (Table 1).

Discussion

The complex biological process and a conducive environment are needed for wound healing to facilitate tissue repair and regeneration. Advanced wound dressings play a significant role in addressing wound healing problems. Its functional, structural and biological properties significantly influence its effectiveness in the healing process.[10, 11] This is reflected in FTIR spectra. The FTIR analysis of BAAS formulations at different BAM-to-alginate ratios (25 %: 75 %, 50 %: 50 % and 75 %: 25 %) revealed significant differences in functional group interactions, structural properties and material characteristics.

All BAAS samples exhibited a broad hydroxyl peak, with intensity increasing proportionally to BA content. Literature supports that broad hydroxyl peaks are characteristic of polysaccharide-protein matrices, suggesting strong molecular interactions and water retention in the BAAS formulations.[12–14] Strong carbonyl and alkyl peaks in BAAS affected the structural stability and mechanical strength, which align with studies on polysaccharide-protein composites to which these interactions contribute. Enhanced alkyl peak intensity observed in BA-rich formulations corresponds to the lipid content of amnion, as described in similar FTIR analyses. The results showed that varying the bovine amnion ratio can significantly affect the material's properties for specific applications, supporting its potential as a multifunctional wound dressing material.

IL-6 has been shown as a multipurpose cytokine that affects the inflammatory phase of wound healing. Excessive IL-6 can lead to chronic inflammation; regulated IL-6 levels are crucial for initiating immune responses and transitioning to the proliferative phase. Incorporating IL-6 modulation into wound dressing products offers several advantages. IL-6 stimulates the recruitment and activation of neutrophils and macrophages at the wound site, essential for clearing debris, pathogens and necrotic tissue. IL-6 facilitates the switch from inflammation to tissue repair by promoting angiogenesis and fibroblast activity.[7, 15, 16]

BAAS combines the biological properties of the BAM with the structural support of alginate to create a composite biomaterial for wound healing. The interaction of IL-6 with other growth factors like bFGF and VEGF is believed to enhance wound healing through tissue regeneration and angiogenesis, which are critical for effective healing. Products that modulate IL-6 can accelerate the transition to the proliferative phase, reducing healing time.[5] Meanwhile, a study by Lee and Mooney showed alginate's inert nature, emphasising its role as a scaffold that does not provoke significant inflammatory responses. This inert property of alginate makes it an excellent carrier for bioactive molecules from BAMs, enhancing its delivery and effectiveness.[17]

The BAM, a key component of the BAAS, is naturally rich in bFGF and other bioactive molecules. The BAM-derived materials showed the potential to release growth factors like bFGF sustainably and their effect on enhancing local cellular activity and accelerating tissue repair**.** The BAM serves as a source for bFGF and stabilises it, protecting it from enzymatic degradation and ensuring its prolonged bioavailability. Its combination with alginate gives synergistic positive by providing a hydrated and biocompatible matrix conducive to the controlled release of growth factors.[8, 17, 18]

The presence of specific functional groups in BAAS is believed to regulate bFGF bioavailability and IL-6 activity by modulating its interactions with cellular receptors and extracellular proteins. Hydroxyl groups contribute to a hydrated microenvironment, which supports cytokine diffusion and stability, preventing excessive IL-6 accumulation that could lead to prolonged inflammation. The carbonyl groups in BAAS, derived from collagen and glycosaminoglycans, may serve as binding sites for bFGF and IL-6 receptors, enhancing controlled signalling. This controlled release mechanism promotes fibroblast proliferation and new tissue formation.[14, 19–21]

In BAAS formulations, the ratio of 50 %: 50 % BA-to-alginate was identified as optimal for maximising bFGF and IL-6 levels, as it balances the

bioactive properties of BAM with the stabilising environment of alginate. These balanced biomaterial composition results were aligned with a Pereira and Bartolo study that emphasised the significance of enhancing growth factor bioavailability.[22] Meanwhile, excessive BA content compared to alginate, as in the 75 %: 25 % formulation, may overwhelm the matrix, leading to reduced stabilisation or release of growth factor and cytokine. The ability of the BAAS to modulate bFGF and IL-6 levels is particularly advantageous in chronic wounds, where growth factor deficiencies are common, as well as in acute wounds requiring rapid angiogenesis and re-epithelialisation. Combining bovine amnion's natural properties and alginate's structural support offers this compound a potential solution for tailored wound dressing.

Conclusion

BAAS formulations demonstrate the interplay between functional groups identified in FTIR spectra and cytokine/growth factor modulation observed in ELISA as evidence that its composition can be tailored to address specific phases of wound healing. Hydrophilic interactions drive anti-inflammatory properties, while alginate enhances the stabilisation and delivery of growth factors.

Ethics

This study was approved by The Ethical Committee in Animal Care and Use Committee of the Faculty of Veterinary Medicine, Airlangga University Surabaya, decision No.2.KEH.1.01.2025, dated _

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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