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Fabrication and Physiochemical Properties of Alginate and Fish Gelatin Biofilm for Wound Healing Application

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Abstract

This research focuses on developing and evaluating alginate and fish gelatin biofilms for wound healing applications. Wound management remains a significant challenge in healthcare, requiring innovative solutions to accelerate healing and minimize complications. The study highlights the biocompatibility, biodegradability, and wound-healing potential of alginate and fish gelatin. Biofilms were fabricated using a casting method with various alginate-to-fish gelatin ratios (100:0, 95:5, 85:15, 80:20, and 75:25) and cross-linking to enhance properties. The resulting films were characterized using FTIR, AFM, SEM, and contact angle measurements. FTIR validated the effective combination of alginate and fish gelatin, whereas SEM and AFM evaluations indicated that a higher fish gelatin concentration enhanced surface shape and roughness. Contact angle assessments showed good hydrophilicity, supporting a moist wound environment. The biofilms demonstrate advantageous characteristics that fulfill the criteria for wound healing materials.

1. Introduction

Wound healing is a complex process involving four overlapping phases, and disruptions in this process can lead to chronic wounds [1]. Alginate, a naturally occurring polymer derived from brown algae, plays a pivotal role in the creation of hydrogels and scaffolds for biomedical applications due to its biocompatibility, biodegradability, low toxicity, and cost-effectiveness. Its structural similarity to cellular matrices makes alginate-based hydrogels particularly effective for wound healing, tissue repair, and drug delivery. Alginate is also ideal for loading bioactive therapeutic compounds, enabling applications in controlled drug delivery, antibacterial treatments, and cancer therapy. By combining alginate with other polymers and bioactive agents, researchers have developed advanced hydrogels and scaffolds with enhanced physiological interactions and multifunctionality [2].

Fish gelatin, derived from fish skin and bones, complements alginate in biofilm formulations by offering mechanical strength, bioactivity, and reduced risk of disease transmission compared to other gelatin sources. It has been shown to accelerate burn wound healing and promote regeneration of bacteria-infected tissues. The combination of alginate and fish gelatin in biofilms provides a scaffold for cell proliferation and bioactivity, enhancing wound healing efficacy. Evaluating the physiochemical properties of these biofilms, such as strength, water absorption, and degradation, is essential for their development as advanced wound dressings. This research highlights the potential of alginate and fish gelatin-based biofilms to revolutionize wound healing therapies, offering innovative solutions for managing chronic wounds and improving patient outcomes [3].

Wound healing is a complex physiological process that occurs in the body following injury or trauma to the skin or other tissues. Several factors can influence the wound healing process, including the size and depth of the wound, the presence of infection or underlying medical conditions such as diabetes, and the individual's overall

health and immune function. Wounds can damage the deeper underlying tissues, causing infection and inflammation, which can compromise the integrity and function of the skin. Gelatin and sodium alginate were assessed for their favourable biocompatibility and extensively utilized in the production of biomaterials, such as wound healing dressings [4]. The effectiveness of these wound dressings as treatments is influenced by the ratio of additional polymers combined with alginate, the type of crosslinkers employed, the duration of crosslinking, the nature of excipients utilized, the integration of nanoparticles, and the presence of antibacterial agents [5]. An effective wound dressing material must exhibit several essential properties, including superior biocompatibility to prevent adverse reactions, biodegradability for safe decomposition over time, adequate mechanical strength for wound protection, high moisture retention to sustain an optimal moist environment, appropriate porosity for gas exchange, and ideally, antimicrobial activity to mitigate infection risk [6]. These synergistic qualities facilitate healing by enhancing cell adhesion, proliferation, and tissue regeneration. This study evaluates the produced alginate and fish gelatin biofilms against critical criteria to ascertain their appropriateness as wound healing materials.

2. Materials and Methods

This paragraph will encompass the complete procedure, including material preparation and characterization methods utilising Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM), and contact angle measurement via Goniometer, followed by a detailed discussion of the results.

2.1 Material Preparation

The materials used in the experiment to fabricate the alginate/fish gelatin (cold water fish skin/250 bloom) biofilm are listed in Table 1, Table 2, and Table 3 show the list of materials used, the composition of sodium alginate, and the composition of fish gelatin.

Table 1 *Composition of Sodium Alginate*

Sodium Alginate(g)	Distilled Water(mL)
1	100

Table 2 *Composition of Fish Gelatin (Cold water fish skin/250 bloom)*

Sodium Alginate solution (w/v %)	Fish Gelatin (Cold water fish skin/250 bloom) (g)	Designation
1	1SAFGC0/1SAFGB0	1SAFGC0/1SAFGB0
	1SAFGC5/1SAFGB5	1SAFGC5/1SAFGB5
	1SAFGC15/1SAFGB15	1SAFGC15/1SAFGB15
	1SAFGC20/1SAFGB20	1SAFGC20/1SAFGB20
	1SAFGC25/1SAFGB25	1SAFGC25/1SAFGB25

Table 3 *Composition of Calcium Chloride*

Calcium Chloride(g)	Distilled Water(mL)
1.11	100

2.2 Methods

To prepare the solution, 1% w/v of sodium alginate was dissolved in 100 mL of distilled water on a magnetic stirring hotplate. To prevent clumping, the alginate powder was gradually added to the water while the stirrer bar mixed the solution continuously for 1 hour. Stirring was maintained until the alginate powder was completely dissolved, forming a homogeneous solution. The alginate solution was then blended with varying volumes (0 mL, 5 mL, 15 mL, 20 mL, and 25 mL) of fish gelatin, cold water and fish skin by stirring for 30 minutes. Each blended solution was carefully poured into three petri dishes, ensuring the solution evenly covered the entire surface area. The samples in the petri dishes were then subjected to direct crosslinking using a 0.1% calcium chloride solution for 15 minutes per sample. The entire procedure was repeated using fish gelatin with a bloom strength of 250.

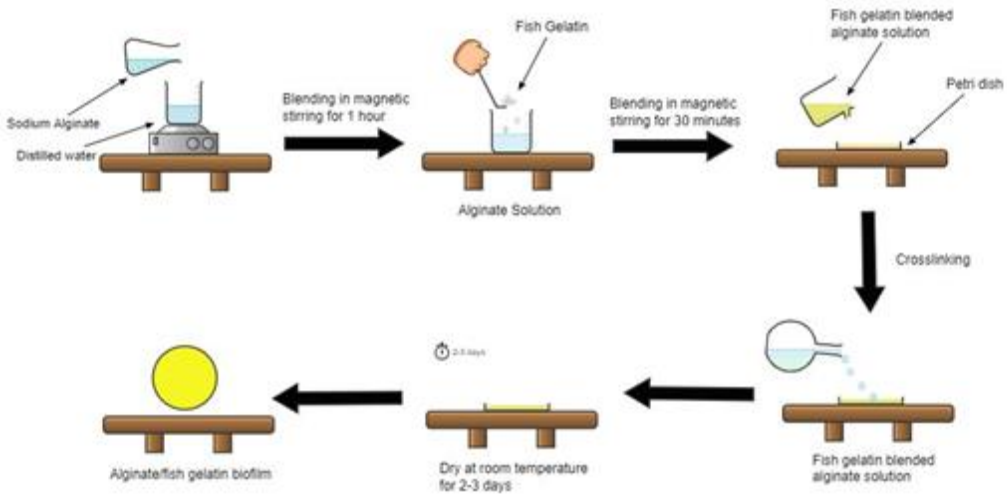

Fig. 1 *Schematic Diagram of Fabrication Process Alginates /Fish Gelatin Biofilm*

Fig. 2 *Diagram of crosslinking process*

2.3 Material Testings

Fourier transform infrared spectroscopy (FTIR) enables the identification of specific functional groups in biomaterials, enabling the assessment of the molecular interactions between alginate and fish gelatin (cold water fish skin/250 bloom) components. It facilitates the identification of interactions between alginate and fish gelatin (cold water fish skin/250 bloom), the analysis of biofilm composition, and the verification of the successful formation of fish gelatin solution into the alginate matrix utilizing the Brand-Agilent Tech/Model-Cary 630 [7].

Research Progress in Mechanical and Manufacturing Engineering Vol. 6 No. 1 (2025) p. 76-87

Fig. 3 *Brand-Agilent Tech/Model-Cary 630 (FTIR)*

The scanning electron microscope (SEM) is used to analyze the surface appearance and microstructural characteristics of alginate/fish gelatin (cold water fish skin/250) biofilms with the SEM apparatus (JSM-7600F/JEOL, Japan) at UTHM [8]. This facilitates the examination of the biofilm's physical characteristics, including porosity, texture, and the distribution of fish gelatin (cold water fish skin/250) particles inside the alginate matrix. This knowledge is essential for evaluating the possible applications of biofilm in areas such as wound healing.

Fig. 4 SEM machine (JSM-7600F/ JEOL, Japan) in UTHM

The XE-100 atomic force microscope (AFM) from Park System Corp, South Korea, is used to evaluate the surface topography, mechanical properties, and structural characteristics of biofilms at the nanoscale. AFM provides high-resolution images and quantitative data regarding the biofilm's structure, roughness, and elasticity. This information is necessary for understanding biofilm growth, consistency, and stability, which can guide applications in wound dressing. AFM specifically assists in evaluating the impact of various treatments or environmental factors on the biofilm's integrity and functionality [9].

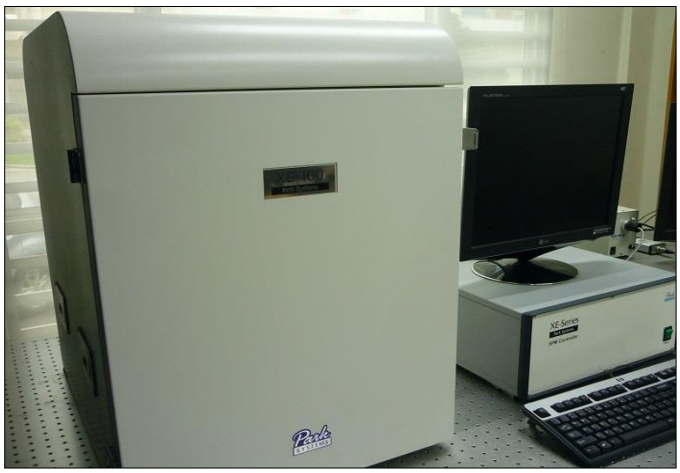

Fig. 5 *the* AFM machine (Park System XE-100 / South Korea) in UTHM

The contact angle measured with a goniometer is an essential method for assessing the surface properties of biomaterials, providing insights into their wettability and interactions with liquids through the VCA Optima Machine (USA) [10]. A low contact angle (< 90°) signifies good wettability, facilitating the easy spreading of liquid, whereas a high angle (> 90°) implies low wettability. The outcome will ascertain if the biomaterial is hydrophobic or hydrophilic.

Fig. 6 *VCA Optima Goniometer (USA) at UTHM*

3. Result and discussion

The results from Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Contact Angle Measurement by Goniometer, and Atomic Force Microscopy (AFM) have been used to characterize and clarify the morphology of biofilms. Fig. 7 illustrates the construction of a pure alginate biofilm absent of fish gelatin, whereas Fig. 8 shows an alginate/fish gelatin biofilm with varying ratios. It has been noticed that increasing the volume of fish gelatin produces a smoother surface with less roughness.

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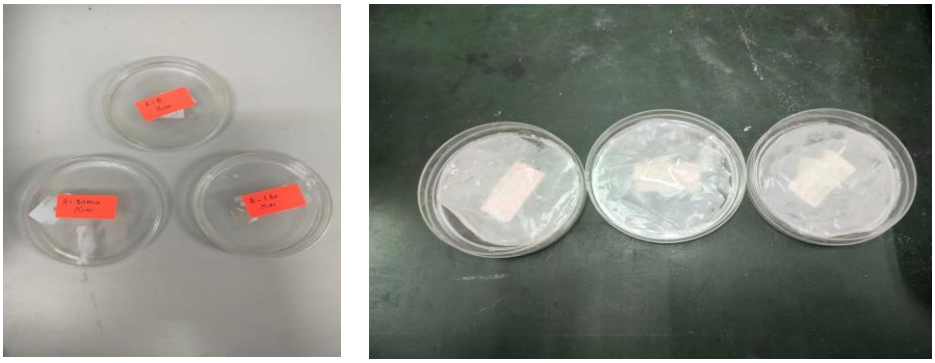
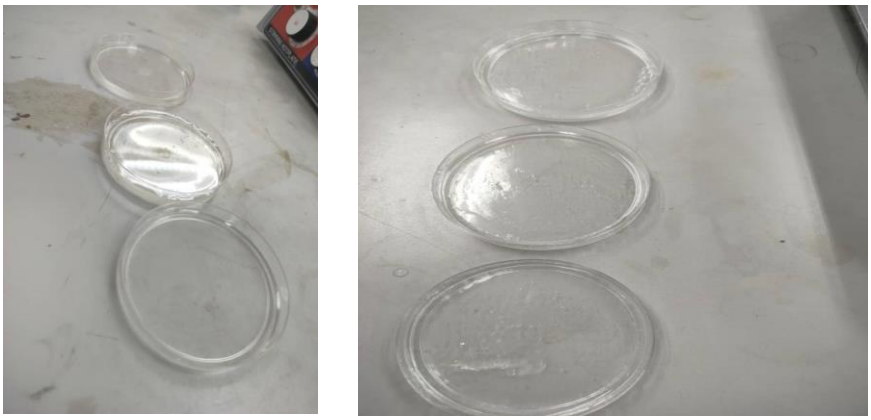
Research Progress in Mechanical and Manufacturing Engineering Vol. 6 No. 1 (2025) p. 76-87



(a) (b)

Fig. 7 *Pure alginate biofilm before drying (a) after drying (b)*



(a) (b)

Fig. 8 *Alginate/fish gelatin with varying ratio 1SAFGC5 (a) and 1SAFGC25 (b)*

3.1 FTIR Bonding Analysis

Avoid hyphenation at the end of a line. Symbols denoting vectors and matrices should be indicated in bold type. Scalar variable names should normally be expressed using italics. Weights and measures should be expressed in SI units. All non-standard abbreviations or symbols must be defined when first mentioned, or a glossary provided.

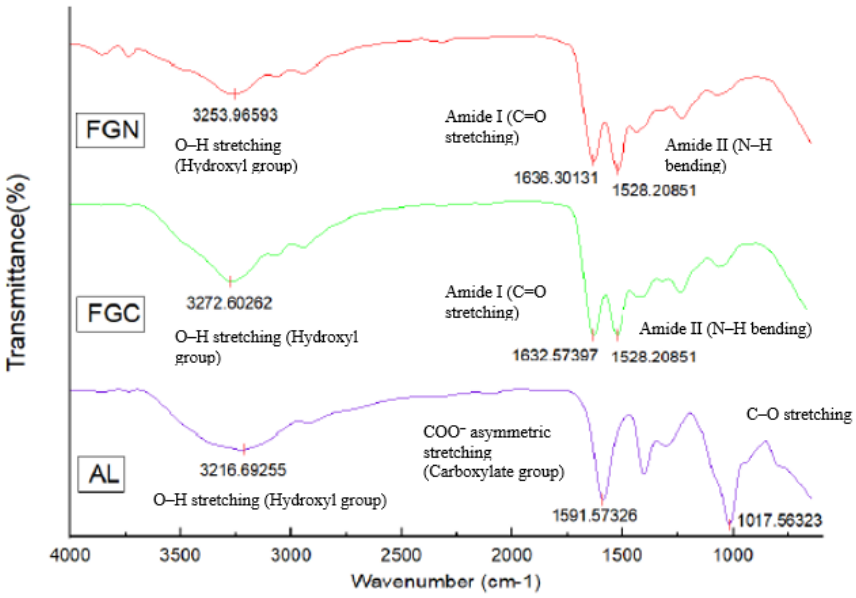


Fig. 9 *FTIR spectroscopy of alginate (AL), fish gelatin cold water fish skin (FGC) and fish gelatin 250 Bloom (FGN)*

According to Fig. 9, the wavenumber range of 3200 to 3400 cm^{-1} for cold fish gelatin exhibits sharper and narrower peaks, indicating improved hydrogen bonding within the gelatin structure. Conversely, fish gelatin (250 Bloom) has wider and less sharp peaks, indicating reduced hydrogen bonding. Within the wavenumber range of 1660 to 1630 cm-1, cold fish gelatin displays stronger peaks, signifying better preservation of its secondary structure. Concurrently, the fish gelatin (250 Bloom) exhibits wider and more displaced peaks, indicating a partial breakdown of the protein's secondary structure. The FTIR spectra acquired facilitate the identification of the functional groups in both alginate and fish gelatin. The table below defines the functional categories according to their respective wave numbers.

Table 4 *Structural Characteristics of Alginate and Fish Gelatin*

Material	Alginate	Fish Gelatin (Cold)	Fish Gelatin 250 Bloom
Main Composition	Polysaccharide (mannuronic acid and guluroic acid).	Protein (denatured collagen), rich in glycine, proline, and hydroxyproline.	Protein (denatured collagen), rich in glycine, proline, and hydroxyproline.
Key Functional Groups	- Carboxylate ($-COO^-$) groups -Hydroxyl(-OH) groups -Ether (C-O-C).	Amide groups (C=O, N-H) -Hydroxyl(-OH) group	-Amide groups (C=O, N-H) -Hydroxyl(-OH) group
FTIR Peaks	-3200-3400 cm^{-1} (O-H stretching) -1590-1610 cm^{-1} (COO^- asymmetric) -1017-1030 cm^{-1} (C-O stretching).	3200-3400 cm^{-1} (N-H/O-H stretching) -1630 cm^{-1} (amide I, C=O) -1520-1530 cm^{-1} (amide II, N-H bending).	5 Similar to cold fish gelatin but with slight variation in FTIR intensity due to higher gel strength (250 Bloom

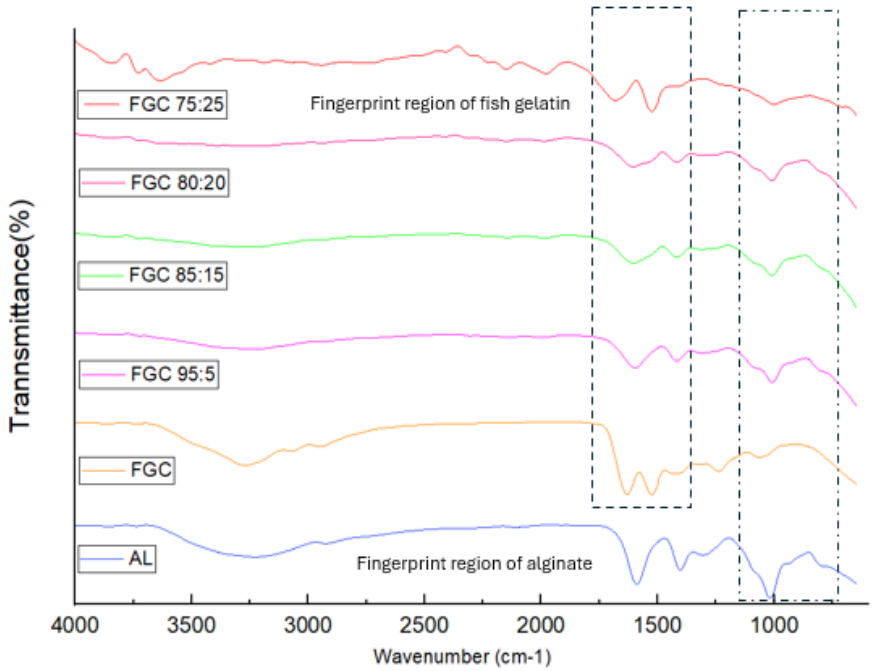


Fig. 10 *FTIR spectra of Al, FGC, FGC 95:5, FGC 85:15, FGC 80;20 and FGC 75:25*

Research Progress in Mechanical and Manufacturing Engineering Vol. 6 No. 1 (2025) p. 76-87

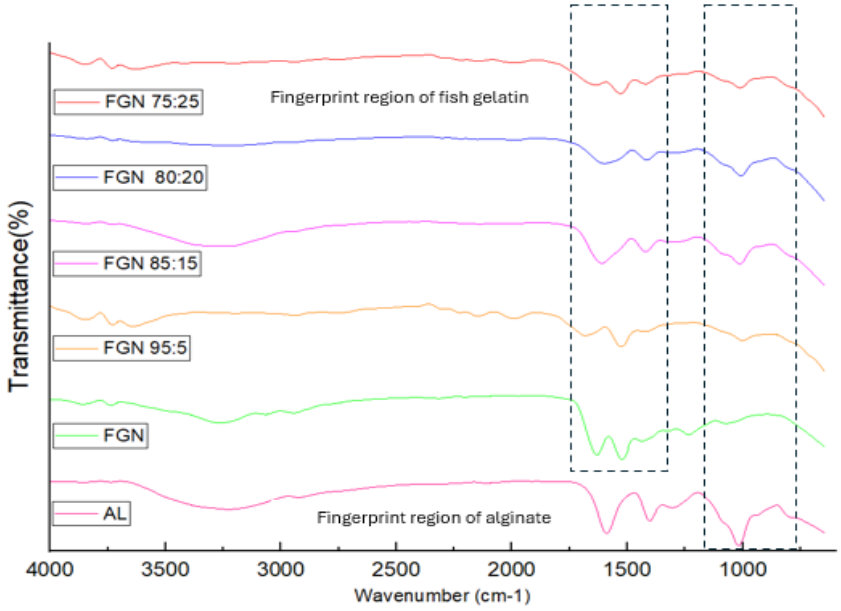


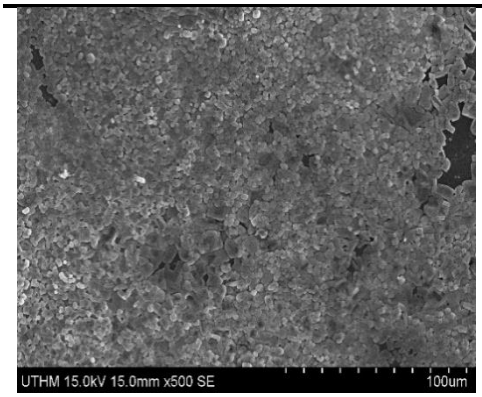
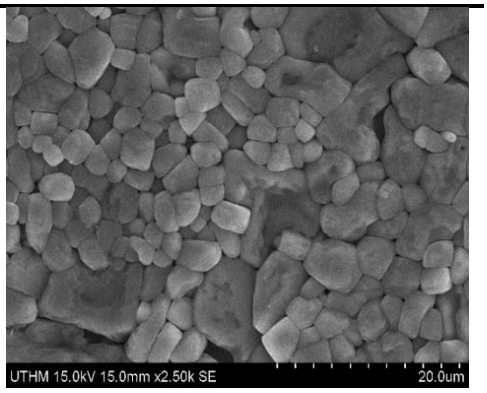
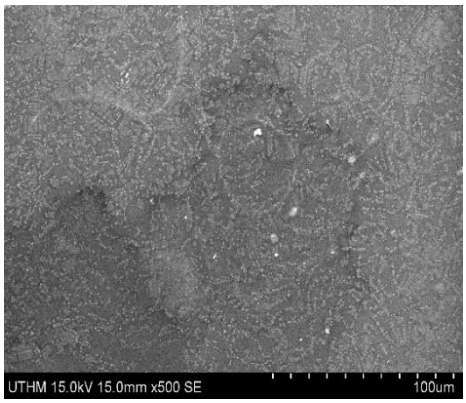
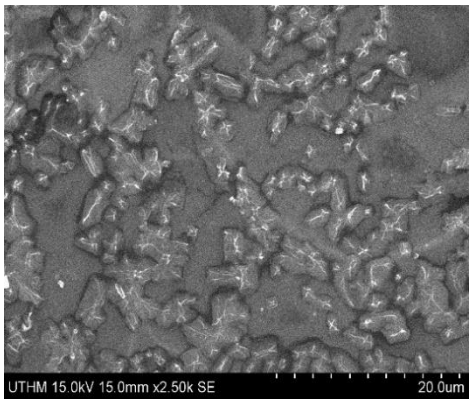
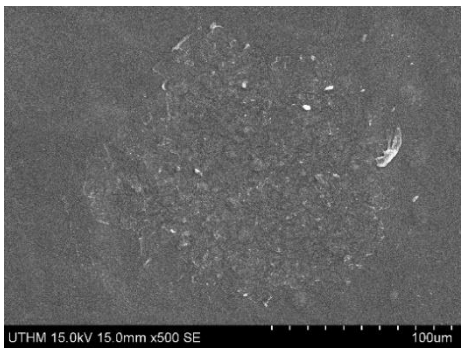
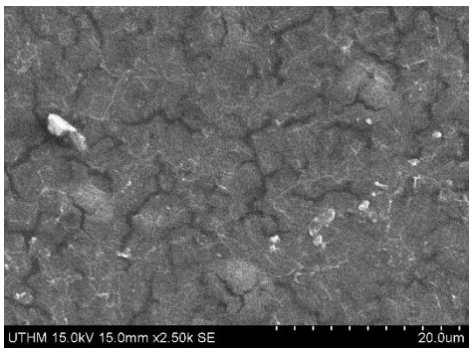
Fig. 11 *FTIR spectra of Al,* FGN, FGN 95:5, FGN 85:15, FGN 80;20 and FGN 75:25

According to Fig. 10 and Fig. 11, the functional group analysis of fish gelatin derived from cold water fish skin, specifically 250 bloom fish gelatin combined with alginate, reveals that the peaks in the range of 3200-3400 cm-1, indicative of O-H stretching, are evident in both alginate and fish gelatin, becoming increasingly pronounced across all samples. The alginate and fish gelatin have been successfully blended. The Amide I peak (1660-1630 cm-1), associated with C=O stretching vibrations, is pronounced in fish gelatin and becomes increasingly prominent in all samples with higher fish gelatin concentration. This produces a more pronounced Amide I peak, reflecting an increased contribution from the protein structure of the fish gelatin. The Amide II peak (1520-1530 cm-1), linked to N-H bending, exhibits a progressive development as the fish gelatin concentration in the mixes increases. This indicates that fish gelatin has been successfully absorbed into the alginate matrix. Both graphs exhibit comparable outcomes, with just minor discrepancies noted.

3.2 Morphology Analysis

In an image of the 1SAFGC0 at a magnification of 500x, the surface seems comparatively smooth and uniform. This signifies that the pure alginate film has no additional fish gelatin particles. No visible pores or irregular surfaces are evident. This smooth morphology indicates the homogeneity of the alginate matrix. At the magnification of 2500x, the film exhibits a dense and compact structure with minimal cracks or voids. The absence of identifiable granular or particle phases indicates that pure alginate forms a tightly compacted matrix. Aside from that, the 1SAFGC5 at a magnification of 500x, the surface exhibits a slight increase in roughness and irregularity relative to the pure alginate film. Small granular structures or clusters are visible, likely attributed to the fish gelatin integration. At the magnification of 2500x the dispersed particles or aggregates of fish gelatin can be observed within the alginate matrix. The matrix starts to show slight porosity and cracks around the regions containing the fish gelatin. Aside from that, the 1SAFGC25 at a magnification of 500x displays a significantly rougher and more porous morphology on the surface. Aggregates or clusters are clearly visible, indicating that increased fish gelatin content has disrupted the alginate's smooth matrix [11]. At 2500x magnification, large cracks, voids, and a highly porous structure are evident. Fish gelatin appears partially distributed within the alginate matrix, leading to distinct boundaries and possible phase separation.

Table 5 *Comparison SEM images for 1% w/v (Alginate and fish gelatin cold water fish skin)*

Magnification of 500x	Magnification of 2500x
	
1SAFGC0	1SAFGC0
	
1SAFGC5	1SAFGC5
	
1SAFGC25	1SAFGC25

Element	Weight%	Atomic%
C K	24.47	44.45
O K	0.67	0.91
Na K	28.71	27.25
Cl K	42.36	26.07
Cr K	0.28	0.12
Cu L	3.51	1.21
Totals	100.00	

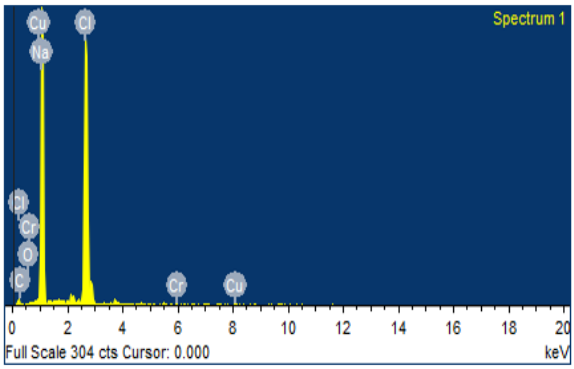


Fig. 12 *Figure Element composition of 1SAFGC0*

Research Progress in Mechanical and Manufacturing Engineering Vol. 6 No. 1 (2025) p. 76-87

Element	Weight%	Atomic%
C K	44.26	56.41
O K	36.84	35.25
Na K	2.37	1.58
Cl K	9.85	4.25
Ca K	6.16	2.35
Cr K	0.51	0.15
Totals	100.00	

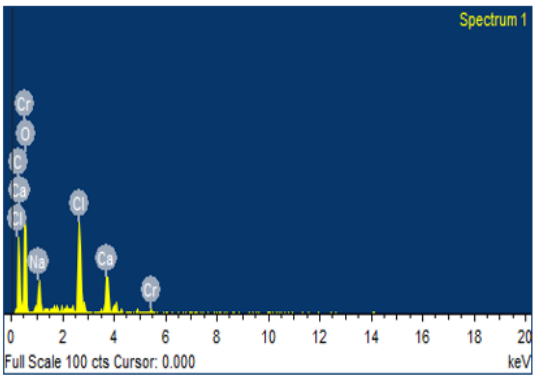


Fig. 13 *Figure Element composition of 1SAFGC25*

3.3 Contact Angle Analysis

The surface wettability of alginate and fish gelatin films was assessed by contact angle measurements. Table 6 presents the contact angle measurements for alginate and fish gelatin biofilms derived from cold water fish skin, including 0 mL to 30 mL of fish gelatin and crosslinked with a 0.1% w/v calcium chloride solution. The assessment of contact angle clarifies the surface characteristics of a hydrogel with varying concentrations of fish gelatin. A higher contact angle signifies decreased hydrophilicity, indicating that the surface is less inclined to engage with water.

Table 6 *Contact angle values for alginate/fish gelatin cold water fish skin biofilm*

Sample	Contact Angle (°)		
	1st	2nd	Average
1SAFGC0	26.00	24.20	251
1SAFGC5	40.50	40.50	40.50
1SAFGC25	83.20	82.60	82.9

(a) **(b)** **(c)**

Fig. 14 *Contact angle of alginate/fish gelatin biofilm (a) 1SAFGC0, (b) 1SAFGC5 and (c) 1SAFGC25*

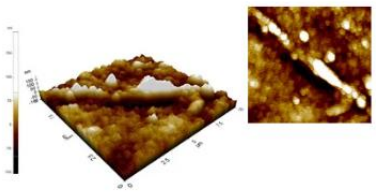
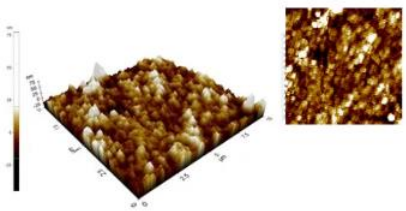
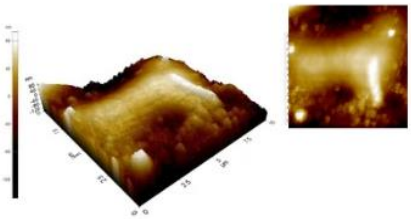
3.4 Surface Roughness Analysis

The surface roughness determined by AFM analysis presents essential information about the topography of the alginate/fish gelatin biofilm samples. Table 7 shows the fluctuation values in surface roughness parameters (Rpv, Rq, and Ra) observed at alginate/fish gelatin cold water fish skin biofilm. The (SAFGC0) has the highest surface roughness. This demonstrates the natural roughness of alginate without of additions. High roughness can influence cell adhesion and protein absorption, both of which are essential for wound healing applications. The (SAFGC5) has the lowest surface roughness. The addition of fish gelatin results in an extra refined surface, advantageous for biofilm applications demanding minimal roughness, particularly in interactions with soft tissues. The more refined surface may additionally affect mechanical stability and the rate of biodegradation. SAFGC25 has moderate surface roughness [11]. An increased amount of fish gelatin results in a more complex surface structure. This suggests that fish gelatin, in specified quantities, creates a unique nanoscale structure suitable for particular uses, including the improvement of cell connection in wound healing.

Table 7 *Surface roughness parameter for alginate/fish gelatin cold water fish skin (FGC) biofilm*

Sample	Surface Roughness		
	Rpv (nm)	Rq (nm)	Ra (nm)
SAFGC0	298.397	34.196	24.331
SAFGC5	122.259	10.175	7.621
SAFGC25	239.837	40.705	33.704

Table 8 *3D and 2D AFM images for alginate/fish gelatin cold water fish skin (FGC) biofilm*

Sample	Fish gelatin cold water fish skin
SAFGC0	
SAFGC5	
SAFGC25	

4. Conclusion

This study effectively produces alginate and fish gelatin biofilms from cold water fish skin/250 bloom by the solution casting technique and examines their physicochemical properties. The investigation explored the dilution of alginate in distilled water at a concentration of 1% w/v, a combination of alginate and fish gelatin derived from cold water fish skin/250 bloom biofilm solutions at various ratios, the fabrication of biofilms via solution casting, cross-linking with 0.1% calcium chloride, and the analysis of physicochemical properties using FTIR to ascertain the differences in bonding of the biofilm with and without fish gelatin from cold water fish skin/250 bloom biofilms.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

This study was planned and designed by [Kirubaneeswary a/p Rethnam @kiru], who also conducted the data analysis. Prof. Dr. Maizlinda Izwana Binti Idris made substantial contributions to the analysis of the results and offered essential changes.

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