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Fabrication and Physiochemical Properties of Chitosan and Fish Gelatin Biofilm for Wound Healing Process

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Abstract

This study explores the development and characterization of a chitosan/fish gelatin biofilm for wound healing applications. Chronic wounds are a growing healthcare concern, especially among elderly and immuno-compromised patients. Conventional synthetic wound dressings often lack biocompatibility and sustainability, highlighting the need for bio-based alternatives. Chitosan, known for its biodegradability, biocompatibility, and antibacterial properties, was combined with fish gelatin, recognized for its compatibility with living tissue and wound-healing potential. The biofilms were fabricated using a solution casting method with varying ratios of chitosan and fish gelatin (100:0, 95:5, 90:10, 85:15 and 80:20) and cross-linked with glutaraldehyde to enhance mechanical stability. Physiochemical properties of the biofilms were analyzed using Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), and contact angle measurements. Fourier Transform Infrared Spectroscopy (FTIR) confirmed successful blending and cross-linking, as evidenced by characteristic peaks associated with amide and hydroxyl groups. Scanning Electron Microscopy (SEM) revealed a homogeneous surface morphology with improved structural integrity as fish gelatin content increased. Atomic Force Microscopy (AFM) showed a reduction in surface roughness, with the root mean square roughness (Rq) decreasing from 64.033 nm in the CH biofilm to 2.546 nm in the CHCF20 biofilm, indicating improved smoothness and compatibility for wound healing. Contact angle measurements indicates that the material is hydrophilic, as evidenced by the significant reduction in contact angles from 87.03° to 83.03° and further to 67.4° with increasing gelatin ratio, showcasing its ability to retain moisture and support healing. This study successfully demonstrates the potential of chitosan/fish gelatin biofilm as an effective, biocompatible, and environmentally sustainable wound dressing.

1. Introduction

In daily life, individuals are often susceptible to sustaining skin wounds, which can cause significant physical and emotional distress. These injuries not only affect well-being but also impose a considerable financial burden on society. Wound healing is a complex biological process through which the body repairs and regenerates damaged tissue. To accelerate this process, specialized gels and creams are frequently applied in clinical settings

[1]. The growing prevalence of chronic, high-risk wounds particularly among vulnerable populations such as the elderly, immunocompromised individuals, and diabetics highlights the increasing need for effective wound treatment solutions [2]. Wound dressings should possess favourable mechanical qualities, be non-toxic, and exhibit biocompatibility. Furthermore, suitable healing materials should facilitate the exchange of gases and mitigate inflammation. Dressing materials commonly utilize synthetic polymers, including poly(lactide-co-glycolide), polyethylene glycol, polycaprolactone, and polyurethane. Nevertheless, these materials possess several constraints, hence preventing their extensive utilization as wound dressings. Polysaccharides and proteins are compatible with the human body and its biological activities. Thus, they have the potential to serve as a perfect substitute for synthetic substances [3][4].

Chitosan has been identified as a desirable substance for use in wound healing treatments because of its distinct biological characteristics such as biodegradability, biocompatibility, and little to no toxicity. Moreover, chitosan has antibacterial properties, hemostatic qualities and it is also mucoadhesive and can enhance the rate of healing of wounds [5]. Chitosan is a powerful material that can be utilized in the field of wound dressing. However, it is recommended that it be blended with other biopolymers. Therefore, combining chitosan with gelatin could be a possible technique for obtaining a novel polymeric material that possesses superior physicochemical properties and possesses a distinct functionality [6].

Gelatin is a polymer that is soluble in water and compatible with living organisms. Fish gelatin extracted from fish skin and or fish bone has gained focus as an initial material in several biomedical applications. Fish gelatin has favourable characteristics such as biocompatibility, biodegradability, hydrophilicity, and water retention ability. Furthermore, fish gelatin is considered an optimal substance for promoting blood clotting and treating wounds due to its effective coagulation effect on platelets [7][8]. By combining these two materials, it is possible to develop a biofilm with enhanced mechanical strength, hydrophilicity, and antimicrobial properties. This paper aims to explore the fabrication process and characterize the physiochemical properties of chitosan and fish gelatin biofilm for wound healing applications.

2. Materials and Methods

The entire process, encompassing material preparation, methods, and characterization using Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscope (AFM), and Contact Angle measurements with a Goniometer, will be detailed in this paragraph, followed by a more in-depth discussion of the results.

2.1 Material Preparation

The materials used in the experiment to fabricate the chitosan/fish gelatin biofilm are listed in Table 1, Table 2, and Table 3 show the composition of chitosan, fish gelatin and chitosan/fish gelatin for biofilm

Table 1 *Composition of chitosan solution*

Chitosan (g)	Acetic acid (mL)
1	100

Table 2 *Composition of fish gelatin solution*

Fish gelatin (g)	Deionized water (mL)
1	100

Table 3 *Composition of chitosan/fish gelatin for biofilm*

Chitosan solution (mL)	Fish gelatin solution (mL)	Label (cold fish gelatin)	Label (fish gelatin 250 Bloom)
100	0	CH	CH
95	5	CHCF5	CHNF5
90	10	CHCF10	CHNF10
85	15	CHCF15	CHNF15
80	20	CHCF20	CHNF20

2.2 Method for biofilm fabrication

The solution casting method was employed to fabricate a chitosan/fish gelatin biofilm. The process began by pouring acetic acid into a beaker and heating it on a hot plate magnetic stirrer. The magnetic stirrer temperature was kept at 60˚C and set at a speed that would create a vortex in the solution. Then, 1 gram of chitosan was gradually added to the acetic acid. The solution was stirred continuously for 1 hour or until the chitosan powder/particles were completely dissolved in acetic acid at a temperature of 60ºC. The chitosan solution will be cooled down to room temperature. 1 gram of fish gelatin (cold water) was mixed with 100ml of deionized water (DI). The process then continued by pouring deionized water into a beaker and heating it on a hot plate magnetic stirrer until a vortex formed. Then, 1 gram of fish gelatin (cold water) was gradually added to the DI to prevent clumping. The solution was stirred continuously for 30 minutes at a temperature of 30˚C until all the mixtures were well mixed. After that, mix the chitosan solution and fish gelatin (cold water) solution together at a concentration of 1 w/t% with different volume ratios of 100:0, 95:5, 90:10, 85:15, 80:20. Then, the mixture was subsequently removed from the magnetic stirrer. The resulting solution was cooled for 10 minutes before being distributed into three petri dishes, each containing 30 ml. To initiate crosslinking, a glutaraldehyde solution of 0.3ml is pipetted into the petri dish, then the petri dish is swirled around to ensure the mixture is well mixed. The specimens are stored in a drying oven at 27˚C until a biofilm is formed. Repeat the same procedure with fish gelatin (250 bloom). The aim of characterizing it was to produce the film, which was then sliced into squares that were around 1 cm x 1 cm in size.

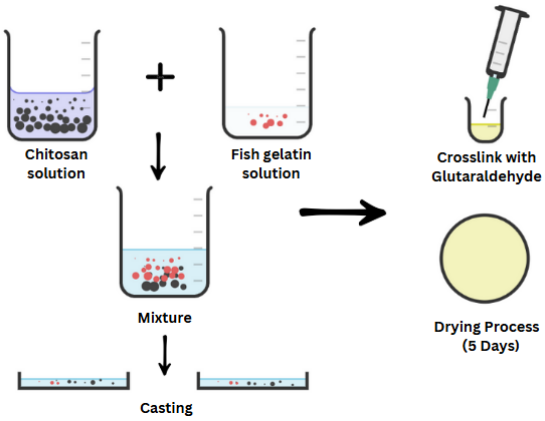

Fig. 1 *Schematic diagram of fabrication process*

Fig. 2 *Diagram of crosslinking process*

2.3 Material Testings

Fourier Transform Infrared Spectroscopy (FTIR) is employed to identify specific functional groups within biomaterials, enabling the assessment of interactions between chitosan and fish gelatin components at the molecular level. This technique aids in analyzing biofilm composition, detecting interactions between chitosan and fish gelatin components, and confirming the successful integration of fish gelatin extracts into the chitosan matrix. The analysis is conducted using the Agilent Cary 630 FTIR Spectrometer.

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Fig. 3 *Agilent Cary 630 FTIR Spectrometer* [6]

Contact angle measurement using a goniometer is an essential method for assessing the surface properties of biomaterials, offering insights into their wettability and interactions with liquids. This is conducted using the VCA Optima Machine (USA). A low contact angle (< 90°) signifies high wettability, allowing liquids to spread easily across the surface, whereas a high contact angle (> 90°) indicates low wettability. These results help determine whether the biomaterial is hydrophilic or hydrophobic.

Fig. 4 *VCA Optima Machine (USA) that was used for Contact Angle Estimation*

The Atomic Force Microscope (AFM), utilizing the XE-100 from Park System Corp, South Korea, is employed to evaluate the biofilm's surface topography, mechanical properties, and structural characteristics at the nanoscale. AFM provides high-resolution images and quantitative data on texture, roughness, and elasticity, which are critical for understanding biofilm formation, adhesion, and stability. This information is particularly valuable for applications in wound dressing, as it enables assessment of the effects of various treatments or environmental conditions on the biofilm's integrity and functionality

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

Scanning Electron Microscope (SEM), specifically the Hitachi SU1510 from Hitachi High Technologies America Inc., USA, is utilized to analyze the surface morphology and microstructural properties of chitosan/fish gelatin biofilms. This technique provides valuable insights into the biofilm's physical characteristics, including porosity, texture, and the distribution of fish gelatin particles within the chitosan matrix. Such information is essential for evaluating the biofilm's suitability for applications like wound healing

Fig. 6 *Scanning Electron Microscope, Hitachi SU1510, Hitachi High Technologies America Inc, USA*

3. Results and Discussion

The findings from FTIR, Contact Angle using a Goniometer, AFM and SEM were utilized to characterize and analyze the morphology of the biofilms. Fig. 7 shows the fabrication of pure chitosan biofilm without adding fish gelatin, while Figure 8 shows the chitosan/fish gelatin biofilm with different ratios. It is observed that increasing the volume of fish gelatin results in a smoother surface with reduced roughness.

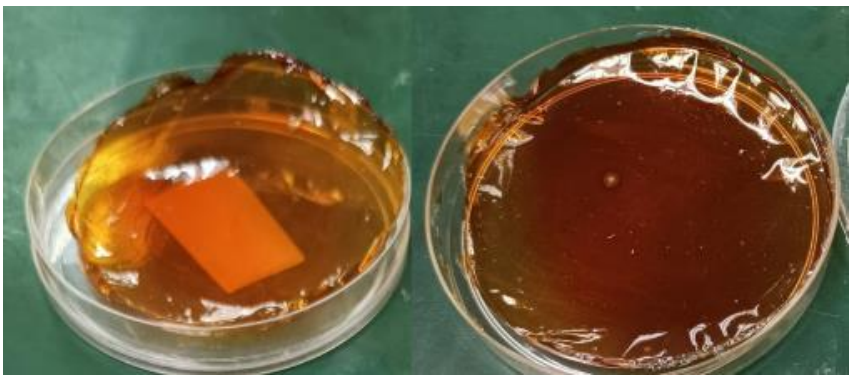

Fig. 7 *Chitosan (CH) biofilm was formed after drying for 5 days in drying oven*

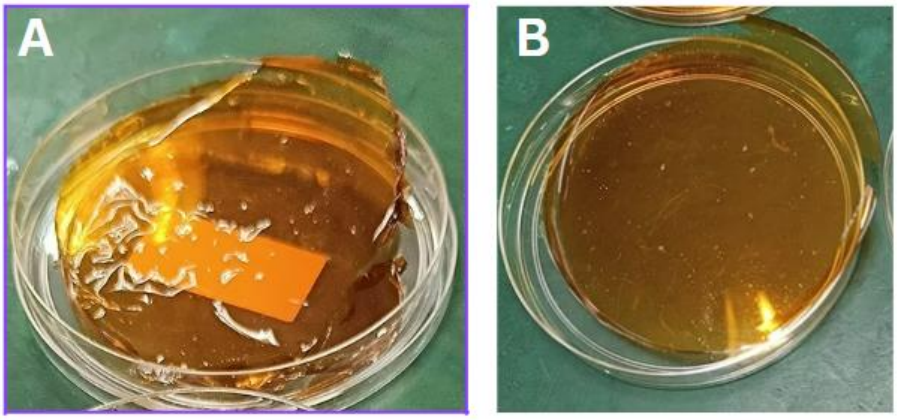

Fig. 8 *Chitosan/fish gelatin biofilm with different ratio CHCF5 (a) and CHCF20 (b)*

3.1 FTIR Bonding Analysis

The measurements for the functional group of the chitosan/fish gelatin biofilm were documented within the range of 4000-600 cm-1. The Fig. 9 below presents the FTIR spectrum of pure substance before any mixing was conducted for the fabrication of the biofilm.

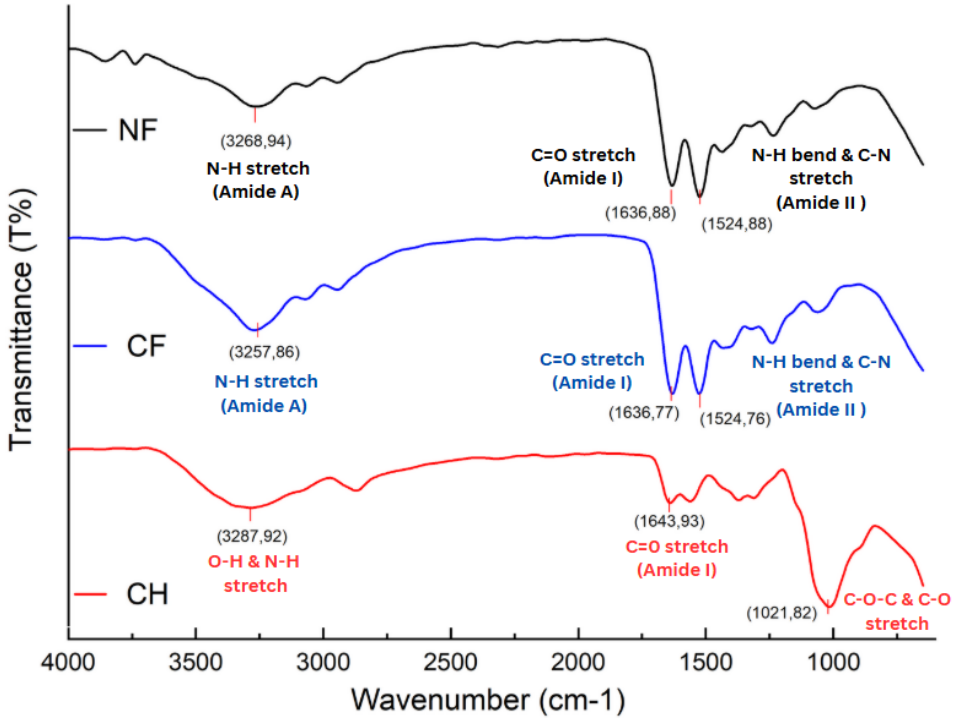

Fig. 9 *FTIR spectroscopy of chitosan (CH), cold fish gelatin (CF) and fish gelatin 250 Bloom (NF)*

Based on Fig. 9, the wavenumber range of 3200 to 3400 cm^{-1} for cold fish gelatin shows sharper and narrower peaks, suggesting stronger hydrogen bonding in the gelatin structure. In contrast, fish gelatin (250 Bloom) shows broader and less defined peaks, reflecting weaker hydrogen bonding. At the wavenumber range of 1660 to 1630 cm^{-1}, cold fish gelatin shows sharper peaks, indicating better retention of its secondary structure. Meanwhile, the fish gelatin (250 Bloom) displays broader and slightly shifted peaks, suggesting a partial loss of the protein's secondary structure. The FTIR spectra obtained allow for the identification of the functional groups present in both chitosan and fish gelatin. The table 4 and 5 below summarizes the functional groups based on their corresponding wave numbers.

Table 4 *Functional peaks of chitosan*

Wavenumber (cm^{-1})	Functional Group	Vibration mode
3200 – 3400	O-H and N-H	Broad stretching vibration (hydroxyl and amine groups overlap)
1660 – 1630	Amide 1 (C=O)	Residual acetyl group vibrations
1100 – 1020	C-O-C and C-O stretching	Polysaccharide backbone vibrations

Table 5 *Functional peaks of fish gelatin*

Wavenumber (cm^{-1})	Functional Group	Vibration mode
3200 – 3400	N-H Stretch (Amide A)	Stretching vibrations (overlaps with O-H)
1660 – 1630	Amide I (C=O)	Strong vibration for peptide bonds
1540 – 1520	Amide II (N-H bend, C-N stretch)	Protein secondary structure

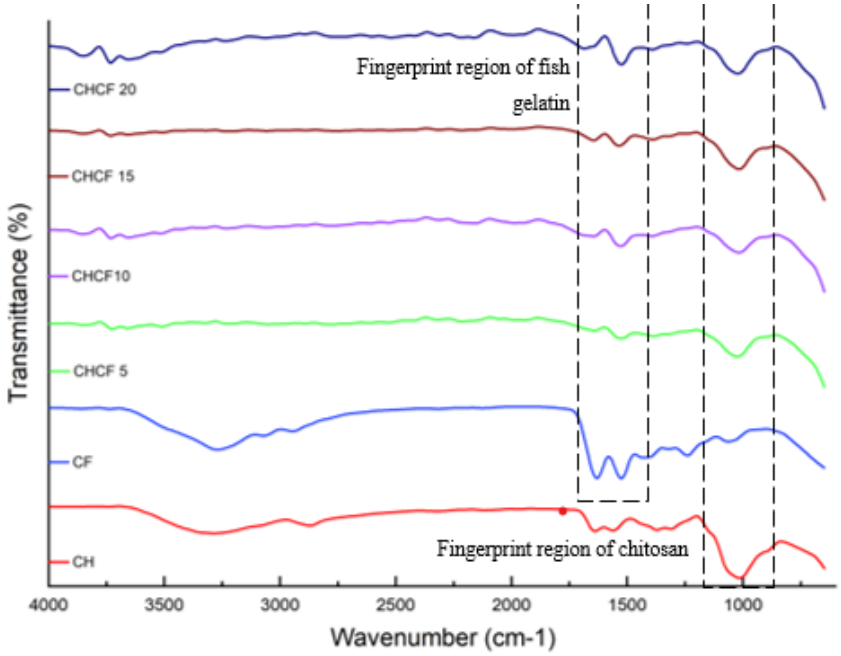

Fig. 10 *FTIR spectra of CH, CF, CHCF5, CHCF10, CHCF15, and CHCF20*

Based on the observations from Fig. 10, The Amide I peak (1660-1630 cm^{-1}), which corresponds to C=O stretching vibrations, is prominent in fish gelatin and becomes more noticeable in all samples as the fish gelatin content increases. This results in a sharper Amide I peak, indicating a greater contribution from the protein structure of the fish gelatin. The Amide II peak (1540-1520 cm^{-1}), associated with N-H bending and C-N stretching, shows a gradual increase in intensity as fish gelatin content rises in the blends. This suggests that fish gelatin is successfully incorporated into the chitosan matrix. Additionally, the C-O-C and C-O-H vibrations observed in the range of 1020–1070 cm^{-1} reflect the polysaccharide backbone of chitosan.

3.2 Contact Angle Analysis

The goniometer contact angle analysis is employed to assess the hydrophilicity of the chitosan/fish gelatin biofilm and to determine whether the addition of fish gelatin influences this property. When fish gelatin is added to chitosan, the contact angle of the biofilm generally decreases. This is because fish gelatin is a hydrophilic material, meaning it has a high affinity for water. The incorporation of fish gelatin increases the hydrophilicity of the biofilm, making the surface more water-absorbent and reducing the contact angle.

Table 6 *Contact angle values for chitosan/fish gelatin biofilm*

Sample	Contact Angle (°)				Standard deviation	Observation
	1st	2nd	3rd	Average		
CH	85.50	90.10	85.50	87.03	2.66	Slightly Hydrophobic
CHCF5	82.10	84.00	83.00	83.03	0.95	Hydrophilic
CHCF20	66.60	65.40	70.20	67.4	2.50	Hydrophilic

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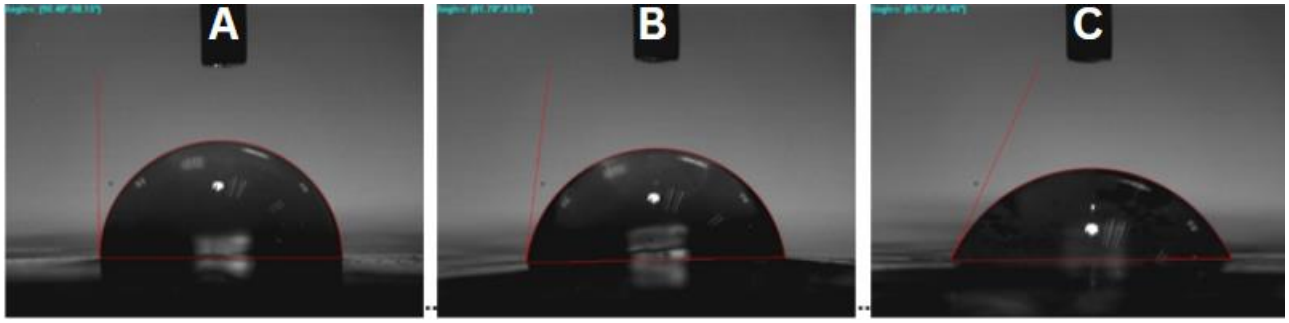
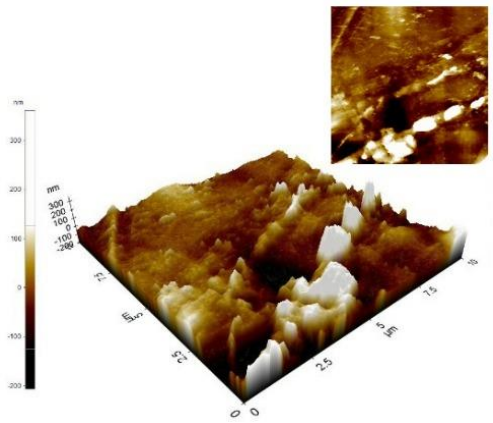
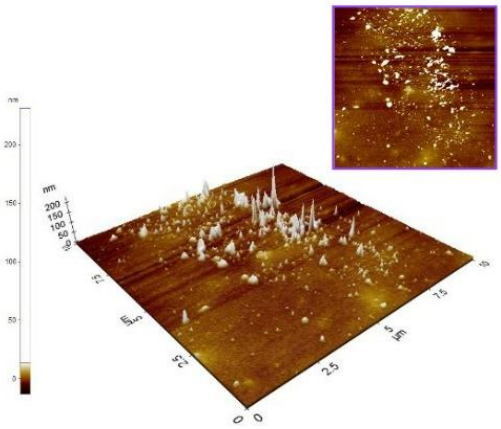


Fig. 11 *Contact Angle of chitosan/fish gelatin biofilm CH (A), CHCF5(B) and CHCF20 (C)*

3.3 Atomic Force Microscope (AFM)

Table 7 illustrates the surface roughness of the chitosan/fish gelatin biofilms with different fish gelatin ratios. The surface roughness in this study is assessed by comparing the mean peak-to-valley value (Rpv), root mean square roughness (Rq), mean roughness (Ra), and average maximum profile height (Rz), as detailed in Table 8.

Table 7 *2D and 3D AFM image of biofilm*

Sample	2D and 3D AFM image
CH	
CHCF5	

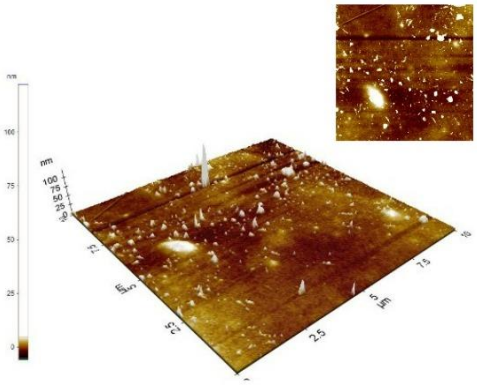
CHCF20	

Table 8 *Surface roughness parameter for chitosan/fish gelatin biofilm*

Sample	Surface Roughness			
	Rpv (nm)	Rq (nm)	Ra (nm)	Rz (nm)
CH	567.934	64.033	43.057	561.143
CHCF5	244.748	6.857	2.918	216.424
CHCF20	128.091	2.546	1.182	118.478

Table 8 shows the surface roughness parameters regarding the chitosan/fish gelatin biofilms with different ratios of fish gelatin. Pure chitosan (CH) biofilm exhibits the highest surface roughness among all the samples. The high surface roughness of the CH biofilm can be attributed to several factors. Firstly, the intrinsic polymeric structure of chitosan forms a rigid and irregular matrix. Additionally, the crosslinking process with glutaraldehyde, while enhancing the mechanical stability of the biofilm, induces surface irregularities during the drying phase of the film [9]. Lastly, the absence of fish gelatin, which could fill voids or smooth the surface, leads to the formation of microcracks or undulations on the biofilm surface.

The addition of fish gelatin, irrespective of its type, significantly reduces the surface roughness of the biofilms compared to pure chitosan. This effect is particularly pronounced in the root mean square roughness (Rq), which decreased from 64.033 nm in the CH biofilm to as low as 2.546 nm in CHCF20. Several factors contribute to this reduction in surface roughness. Firstly, fish gelatin contains hydrophilic functional groups such as -COOH and -NH2, which interact with the hydroxyl (-OH) and amino (-NH2) groups in chitosan[10]. This interaction enhances blending and reduces phase separation. Secondly, fish gelatin molecules fill voids within the chitosan matrix, leading to a more homogeneous film structure. Lastly, fish gelatin exhibits a plasticizing effect, softening the chitosan matrix and reducing its rigidity, which facilitates smoother film formation during the drying process.

3.4 Morphology Analysis

Fig. 12 provides a comparison of the surface morphology for CH, CHCF5 and CHCF20 at magnifications of 2000x. All images include a scale bar representing 20 µm. The SEM pictures of pure chitosan (CH) biofilm show a smooth, uniform surface without visible cracks, crevices, or irregularities. This indicates a well-fabricated and homogeneous biofilm. The optimized concentration of 0.3 ml glutaraldehyde ensured sufficient crosslinking without over-hardening the film, which could otherwise result in brittleness or uneven morphology. The even and consistent surface signifies improved structural integrity and uniformity [11]. The surface morphology of the biofilms exhibited considerable changes depending on the fish gelatin concentration. For CHCF5 biofilm, localised aggregation found at 2000x magnification suggests minimal phase separation, perhaps resulting from inadequate blending or drying irregularities. Conversely, CHCF20 biofilm exhibited a rough surface characterised by visible ridges and clusters but 2000x magnification showed a compact interpenetrating network of chitosan and fish gelatin. The increased roughness and aggregation at higher fish gelatin concentrations indicate its hydrophilic characteristics, which affect drying and crosslinking.

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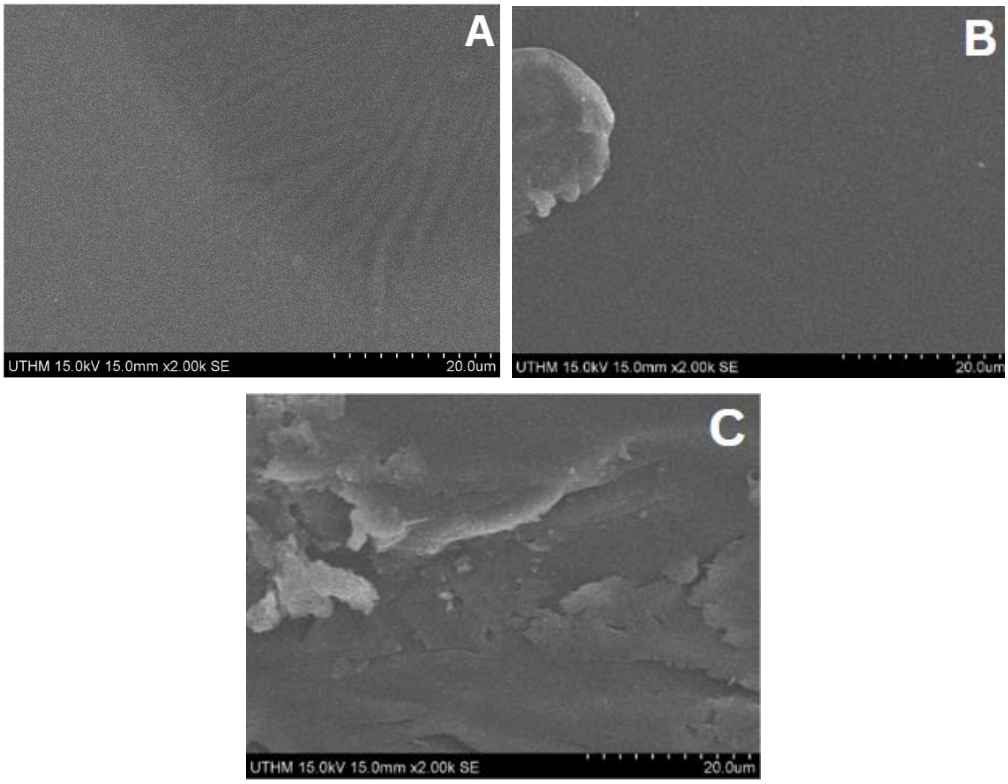
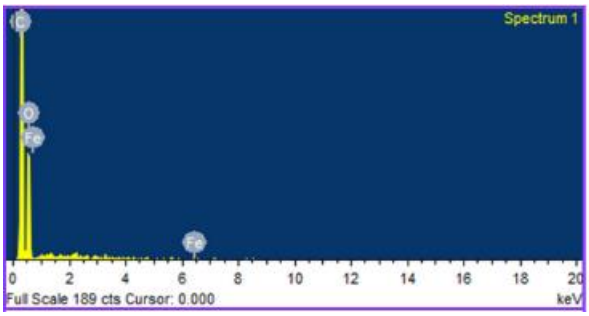
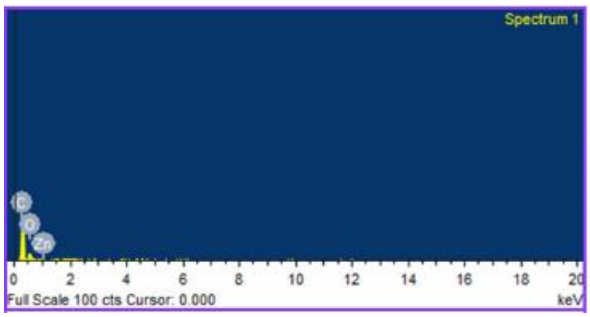


Fig. 12 *SEM image of chitosan/fish gelatin biofilm CH (A), CHCF5 (B) and CHCF20 (C) at magnification of 2000x (scale bar 20 µm)*



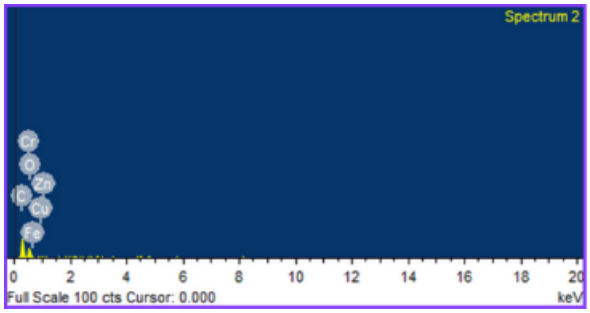
Element	Weight%	Atomic%
C K	58.47	65.30
O K	41.32	34.65
Fe K	0.21	0.05
Totals	100.00	

Fig. 13 *List of Element Composition of CH biofilm*



Element	Weight%	Atomic%
C K	66.97	73.69
O K	31.47	26.00
Zn L	1.56	0.31
Totals	100.00	

Fig. 14 *List of Element Composition of CHCF5 biofilm*



Element	Weight%	Atomic%
C K	53.13	61.32
O K	43.90	38.03
Cr K	0.13	0.04
Fe K	0.02	0.00
Cu L	1.86	0.41
Zn L	0.95	0.20
Totals	100.00	

Fig. 15 *List of Element Composition of CHCF20 biofilm*

The chitosan/fish gelatin biofilm that had been cross-linked with glutaraldehyde was examined using energy dispersive X-ray scanning electron microscopy (EDX) to determine its element composition. Chitosan, fish gelatin and glutaraldehyde are just a few examples of materials that may be quickly and easily analyzed for their element composition using energy dispersive X-ray technology [12,13]. Fig. 13, Fig. 14, and Fig. 15 show the existence of element composition in the chitosan/fish gelatin biofilms crosslinked with glutaraldehyde at a concentration of 0.3mL. The lower atomic percentages of Zn L, Cr K, Fe K, and Cu L have no significant effect on the samples, as their minimal presence is evident in the EDX analysis.

4. Conclusion

The goals of this research were successfully met, focusing on the fabrication of chitosan/fish gelatin biofilm through the solution casting method. Characterization and testing were conducted, including FTIR, SEM, AFM, and contact angle measurements, to explore the physiochemical properties of the chitosan/fish gelatin biofilm. Multiple efforts were made to determine the most effective method for producing the biofilm. Based on these findings, it can be concluded that the optimal sample for this fabrication and is effective for wound healing relies on successful fabrication, with all tests aligning well with its characteristics for wound healing. This study successfully demonstrated the potential of chitosan and fish gelatin biofilms as innovative wound-healing materials. Based on the characterization results, the chitosan/fish gelatin biofilm meets several essential requirements for wound healing materials. FTIR analysis confirmed the presence of amide and hydroxyl groups, indicating successful blending of the two biopolymers. SEM images showed a more uniform surface with increased gelatin content, which supports better cell adhesion. AFM analysis revealed a significant reduction in surface roughness (from 64.033 nm in CH to 2.546 nm in CHCF20), enhancing surface compatibility with skin tissue. Contact angle measurements indicated increased hydrophilicity (from 87.03° to 67.4°), supporting moisture retention—a critical factor in wound healing. Furthermore, both chitosan and fish gelatin are biocompatible, biodegradable, and have natural antibacterial effects. These findings suggest that the fabricated biofilm is a strong candidate for use as an effective and environmentally friendly wound dressing material.

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Conflict of Interest

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

Author Contribution

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