MALAYSIAN JOURNAL OF MICROSCOPY

Journal homepage: https://malaysianjournalofmicroscopy.org

RESEARCH ARTICLE

PHYSICOCHEMICAL AND SURFACE CHARACTERIZATION OF GLUTARALDEHYDE-CROSSLINKED FISH GELATIN/CHITOSAN BIOFILMS FOR WOUND DRESSING APPLICATIONS

Noor Athirah Aida Noor Rizan @ Noor Jehan[1], Maizlinda Izwana Idris[1,2,*], Mohamad Ali Selimin[2,3]

[1]Department of Manufacturing Engineering, Faculty of Mechanical & Manufacturing Engineering, University Tun Hussein Onn Malaysia, Batu Pahat, 86400 Parit Raja, Johor, Malaysia.
[2]Centre of Bioactive Materials (BioMa) centre of Research, Faculty of Mechanical and Manufacturing Engineering, University Tun Hussien Onn Malaysia, Batu Pahat, 86400 Parit Raja, Johor, Malaysia.
[3]Department of Production and Operations Management, Faculty of Technology Management and Business, University Tun Hussein Onn Malaysia, Batu Pahat, 86400 Parit Raja, Johor, Malaysia.

Abstract. This work focuses on the fabrication of wound healing materials from natural fish gelatin and a bio-based polymer chitosan. The main aim was to develop a biocompatible film with excellent physicochemical properties as protective layers for effective wound healing treatments. In this work, fish gelatin was selected for its low immunogenicity and film-forming ability, while chitosan enabled to improve surface wettability and biocompatibility. The fish gelatin/chitosan biofilms were prepared using the solution casting method with different chitosan and black tilapia fish skin gelatin ratios (FC-100:0, FC-95:5, and FC-80:20) and subsequently crosslinked with 0.6 mL glutaraldehyde (0.25 % v/v working concentration) to enhancing the structural stability without inducing brittleness. All prepared samples were analysed using Fourier Transform Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM) and contact angle measurement via goniometer. FTIR analysis, it was indicated strong molecular interactions between gelatin and chitosan through O–H and N–H stretching bands. Surface roughness analysis by AFM showed that FC-95:5 produced the most uniform surface morphology (Ra = 2.479 nm, Rq = 3.530 nm), while FC-80:20 exhibited the highest surface roughness (Ra = 11.943 nm, Rq = 16.928 nm). In addition, contact angle measurements confirmed hydrophilic surface wettability across all formulations, with FC-80:20 recording the lowest average contact angle of 65.9 °, indicating the greatest water affinity among the samples. In conclusion, the fish gelatin/chitosan biofilms exhibited the most promising physicochemical and surface characteristicsas a natural and sustainable foundation for potential wound dressing applications.

Keywords: Fish gelatin, chitosan, wound healing, wound dressing.

Article Info

Received 15 February 2026
Accepted 22 April 2026
Published 8 June 2026

***Corresponding author: izwana@uthm.edu.my**

ISSN: 1823-7010, eISSN: 2600-7444

1. INTRODUCTION

Biomaterials have revolutionized the field of regenerative medicine, particularly in wound healing applications. These materials enhance tissue development, repair, and angiogenesis, making them crucial for the regeneration of ruptured tissues in both humans and animals. Wound healing is a complex biological process involving multiple stages, including haemostasis, inflammation, proliferation, and tissue remodeling. Conventional wound dressings such as bandages, sponges, and powders have been widely used to protect wounds and provide an optimal environment for healing. However, these conventional dressings often face limitations, including poor infection control and insufficient promotion of healing [1]. Chronic wounds present significant challenges that traditional dressings struggle to address effectively. The complexity of the healing process and the need for more effective solutions have driven the development of new and advanced wound care strategies that leverage biomaterials to mimic the extracellular matrix and fight infections. These advancements aim to transform wound care into an intelligent, adaptive process that responds to patient-specific needs, potentially leading to personalized scar-free healing technologies [2].

The latest literature highlights significant progress and evolution in the field of wound healing materials, particularly the use of natural polymers, such as gelatin from fish and chitosan. Chitosan, a naturally occurring biopolymer formed by removing acetyl groups from chitin, has attracted the attention of people nowadays due to its beneficial biological properties such as biocompatibility, biodegradability, antibacterial effects, and promotion of skin regeneration [3]. Studies have shown that chitosan-based materials, such as hydrogels, fibers, membranes, films, and sponges, can significantly enhance the wound healing process by promoting hemostasis, reducing inflammation, and facilitating the proliferation of granulation tissue .The combination of chitosan with other polymers, such as gelatin has also been found to improve the overall healing activity, as evidenced by increased wound contraction and enhanced mechanical properties of the composite films [4]. The integration of bioactive agents, growth factors, and stem cells into these dressings has also been explored to enhance their efficacy in treating chronic wounds [5]. Despite these advancements, challenges remain in effectively managing chronic wounds, necessitating ongoing research to address performance, risk-benefit balance, and cost-effectiveness.

Despite the availability of various wound-healing materials, a significant gap exists in the utilization of marine-derived biomaterials, particularly fish gelatin, in wound management. Fish gelatin derived from fish biowaste offers several advantages over mammalian gelatin, including higher biocompatibility, lower immunogenicity, and the absence of risks associated with transmissible diseases such as bovine spongiform encephalopathy [6,7]. Additionally, fish gelatin is economically and environmentally beneficial to making it a promising alternative for biomedical applications [8]. However, despite these advantages, the use of fish gelatin in wound healing remains underexplored, with limited commercial products available. This knowledge gap highlights the need for further research to fully understand the potential of fish gelatin in wound healing and to develop effective, fish gelatin-based wound healing materials.

To address the limitations of fish gelatin and enhance its wound healing properties, combining it with chitosan, which is a natural polysaccharide derived from chitin, presents a promising solution. Chitosan is well-known for its biocompatibility, biodegradability, antimicrobial properties, and ability to promote tissue regeneration [4]. When combined with fish gelatin, chitosan can help balance the degradation rates of both materials, as chitosan degrades slowly while gelatin degrades rapidly [5]. This combination can create a more stable and effective wound dressing material. Studies have shown that chitosan-gelatin composite films exhibit improved mechanical properties, such as increased thickness, folding endurance, water absorption capacity, and tensile strength, compared to chitosan or gelatin alone [9]. Additionally, the combination of chitosan and gelatin has been found to enhance wound contraction and promote faster wound healing in vivo [10,11]. Therefore, the synergistic effects of chitosan and fish gelatin can potentially lead to the development of superior wound healing materials.

To enhance the structural integrity and functional stability of the composite biofilms, glutaraldehyde was selected as the crosslinking agent in this study. Although glutaraldehyde is known to be cytotoxic at high concentrations, its use in biopolymer crosslinking at low working concentrations has been widely documented in the literature. At the concentration employed in this study (~0.25 % v/v), glutaraldehyde has been demonstrated to achieve effective and stable crosslinking of gelatin matrices without inducing cytotoxic effects, with cell viability values of 96–105 % recorded for gelatin-based systems crosslinked at this concentration [7]. While, non-toxic alternatives such as genipin and carbodiimide-based crosslinkers (EDC/NHS) are available but higher cost and limited commercial availability make less practical for preliminary fabrication and characterisation studies of this nature. Genipin, in particular, while biocompatible, introduces blue-coloured crosslinks that may complicate optical and spectroscopic characterisation of the films [10]. Considering the low working concentration used and these practical limitations, glutaraldehyde was considered a suitable crosslinking agent for this investigation.

Surface roughness is a critical surface characteristic of wound dressing biomaterials that directly influences surface wettability, protein adsorption, bacterial adhesion, and the overall interaction of the material with the wound environment [11]. Surface topography has been shown to affect protein deposition, microbial adhesion, and cell behaviour at the biomaterial interface, making it an essential parameter in the evaluation of wound dressing films [11]. In studies involving biopolymer-based films, Karydis-Messinis et al. [5] observed that increasing fish gelatin concentration in chitosan-fish gelatin hydrogel membranes led to rougher surface morphologies, indicating that biopolymer composition directly control surface texture. Similarly, D'souza et al. [9] demonstrated that formulation changes in chitosan/gelatin composite films introduced measurable surface irregularities. At the nanoscale, AFM evaluation of a neat chitosan-gelatin composite biofilm fabricated by solution casting recorded a maximum roughness of 29.4 nm, attributed to strong integration between chitosan and gelatin through hydrogen bonding and electrostatic interactions [10]. These values serve as a reference baseline for contextualising the AFM roughness parameters obtained in the present study.

The primary aim of this research is to fabricate and characterize films composed of black tilapia fish gelatin and chitosan using the solution casting method. By investigating the molecular interactions between fish gelatin and chitosan through Fourier Transform Infrared Spectroscopy (FTIR), evaluates the surface topography and nanoscale roughness of the biofilms via Atomic Force Microscopy (AFM), and assesses the surface wettability of the films through contact angle measurements using a goniometer. The goal is to provide a comprehensive physicochemical and surface characterisation of the fabricated biofilms across three chitosan-to-gelatin volume ratios (FC-100:0, FC-95:5, and FC-80:20), enabling identification of the most promising formulation for potential wound dressing applications. The successful development and characterisation of these fish gelatin/chitosan biofilms represent a foundational step toward establishing a natural, sustainable alternative to conventional wound dressing materials, while reducing reliance on mammalian gelatin sources.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in the experiment to fabricate the chitosan/fish gelatin films included chitosan (Poly(D-glucosamine), deacetylated chitin; molecular weight ~50–190 kDa; degree of deacetylation ≥75 %, Sigma-Aldrich, St. Louis, MO, USA). Acetic acid solution with 1.0 mol/L (1N) brand Bendosen from Orioner Hightech Sdn Bhd. Glutaraldehyde solution (25 % v/v aqueous stock, glutaric dialdehyde, Pentane-1,5-dial) from Sigma–Aldrich (St. Louis, MO, USA) and distilled water. The fish gelatin powder was acid-extracted (Type A gelatin) from black tilapia fish skin sourced from the local market and extracted manually. The extraction process was carried out using an acid pre-treatment method. Cleaned skins were soaked in hydrochloric acid, HCl (Brand EMORY) solutions with a concentration of 0.03M for 4 hours. After acid treatment, distilled water was used to rinse the skin until the pH was neutral. The extraction procedure was then carried out for 4 hours at 45 degrees

in a water bath. The solutions were filtered through Whatman filter paper No. 4 (Sigma Aldrich, St. Louis, Mo, USA) and then allowed to dry in a universal oven (Model UF750, Memmert GMbH + Co.KG, Germany) set to 70 °C for 48 hours after extraction. After that, the dried film was blended to create fish gelatin powder.

2.2 Preparation and Fabrication of Chitosan/Fish Gelatin Film

The chitosan/fish gelatin composite films were produced by solution casting, a standard methodology for forming biopolymer films. The fabrication procedure involved by preparing separate polymer solutions, mixing at different ratios, crosslinking, casting into petri dish and drying process [12]. The sequence of steps is illustrated in Figure 1.

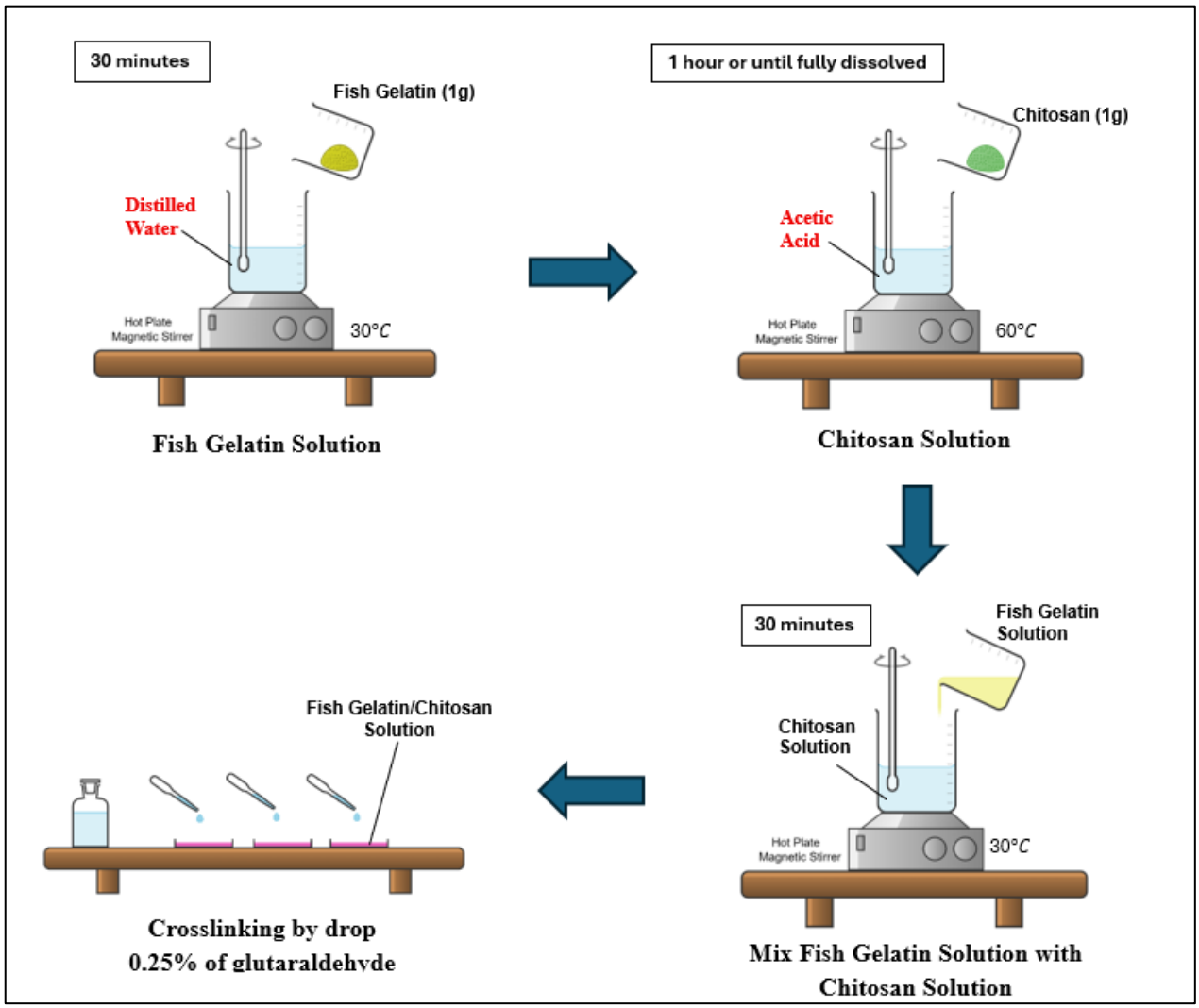


Figure 1: Schematic diagram of the fabrication process of chitosan/fish gelatin composite films

First, a fish gelatin solution was prepared by dissolving 1 g of fish gelatin powder in 100 ml distilled water. The mixture was heated to 30 °C and stirred continuously using a magnetic bar for 30 minutes on a hot plate magnetic stirrer to ensure full homogeneous solution. In parallel, a chitosan solution was formulated by dissolving 1 g chitosan powder in 1.0 mol/L acetic acid and stirring at 60 °C for about one hour until complete dissolution, then allowed to cool to room temperature. After both solutions were prepared, blending was made at specific volume ratios (chitosan:fish gelatin) of 100:0, 95:5 and 80:20. These ratios were selected based on preliminary screening and ranges reported in the

literature for chitosan/gelatin composite films [6, 10] enabling a progressive evaluation of the influence of increasing gelatin content on the physicochemical and surface properties of the biofilms.

For each formulation, the gelatin solution was gradually added into the chitosan solution, under continuous stirring at 30 °C for 30 minutes to promote even mixing and interaction between both compositions. Then, 30 mL of each blended mixture was poured into petri dishes; three replicates were made for each ratio. The filled dishes were left at ambient temperature (25 °C) for approximately 30 minutes to allow surface bubbles to escape and mixture to stabilize prior to crosslinking. Crosslinking was performed directly in the petri dishes. The glutaraldehyde stock solution (25 % v/v) was diluted with distilled water to yield a working concentration of approximately 0.25 % v/v, representing a 100-fold dilution from the stock. A volume of 0.6 mL of the diluted glutaraldehyde solution was applied in a dropwise manner onto the surface of each film-forming solution, and the dishes were gently swirled to promote even distribution across the polymer matrix. This surface-application approach was adopted following the protocol reported by Kim et al. [13].

It is acknowledged that dropwise surface addition may produce a crosslinking gradient, with a more densely crosslinked upper layer relative to the bulk; this limitation is considered in the interpretation of the results. Post-fabrication washing and neutralisation were not performed in this study. This decision is supported by evidence that glutaraldehyde at concentrations of 0.25–0.5 wt% does not produce cytotoxic residues within the polymer matrix; cell viability values of 96–105 % were recorded for gelatin-based systems crosslinked at 0.25 wt% glutaraldehyde, indicating full cellular compatibility [7]. Cytotoxic effects from unreacted glutaraldehyde have been reported only at substantially higher concentrations, where residual monomers are released in quantities sufficient to compromise cell viability [12,14]. Following crosslinking, the films were dried at 25 °C for two days to allow complete solvent evaporation without introducing thermal stress, thereby reducing the risk of cracks or defects in the film. Once dry, the films were gently peeled from the petri dishes and cut into uniform squares (1.5 cm × 1.5 cm) for further physicochemical and mechanical analyses.

2.3 Characterization of Chitosan/Fish Gelatin Film

FTIR was used to identify specific functional groups within biomaterials, allowing to assess the interaction of the functional group between fish gelatin and chitosan at the molecular level. It helps in identifying interactions between fish gelatin and chitosan components and analyzing the composition of biofilms. This chemical analysis was carried out using a Fourier transform infrared (FTIR) spectrophotometer (Model 100 series, Perkin Elmer) in the range of 500–4000 cm^{-1}. During the scanning process, controlled pressure is applied to enhance the frequency response. This equipment will facilitate the identification of chemical bond vibrations and generate distinct frequency patterns in the resulting graph.

AFM was applied to assess biofilm surface topography, mechanical properties, and structural aspects at the nanoscale. Atomic force microscope (AFM), XE-100 from Park System Corp, South Korea gives high-resolution images and quantitative data on the biofilm's texture, roughness, and elasticity. This information is crucial for understanding biofilm formation, adhesion, and stability, which can inform applications in wound dressing. AFM helps in assessing the effects of different treatments or environmental conditions on the biofilm's integrity and functionality.

Lastly, the contact angle measurement using a goniometer is an essential technique for assessing the surface properties of biomaterials, particularly their wettability and liquid interaction behavior. In this study, measurements were performed using a VCA Optima Goniometer (AST Products Inc., USA). A smaller contact angle (<90 °) indicates greater wettability, where the liquid spreads easily across the surface, whereas a larger angle (>90 °) reflects lower wettability. This analysis helps determine whether the biomaterial exhibits hydrophilic or hydrophobic characteristics. During the procedure, liquid droplets were placed on different areas of the film surface, and the standard deviation was calculated to ensure the precision and reliability of the obtained data

3. RESULTS AND DISCUSSION

3.1 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of FC-100:0, FC-95:5, and FC-80:20 films shown in Figure 2 illustrate the characteristic absorption bands and functional groups of each biofilm formulation. In FTIR spectroscopy, transmittance (%) reflects the relative absorption intensity at each wavenumber, where lower transmittance indicates stronger molecular absorption. The chitosan crosslinked with glutaraldehyde sample FC-100:0 film showed a broad absorption band at approximately 3246 cm^{-1}, corresponding to O–H and N–H stretching vibrations (Amide A, transmittance 92.65 %), indicating extensive hydrogen bonding within the chitosan network. When glutaraldehyde is used as a crosslinking agent, it reacts with the amino groups of chitosan to form imine (C=N) linkages through a Schiff-base reaction. The absorption bands at 1643 cm^{-1} (Amide I, C=O stretching, transmittance 90.20 %) and 1550 cm^{-1} (Amide II, N–H bending coupled with C–N stretching, transmittance 87.08 %) signify residual N-acetyl groups in chitosan alongside the formed imine linkages. The Amide B band at approximately 2929 cm^{-1} (transmittance 95.43 %) is attributed to asymmetric stretching of C–H and CH_2 groups with N–H contributions, characteristic of the chitosan backbone.

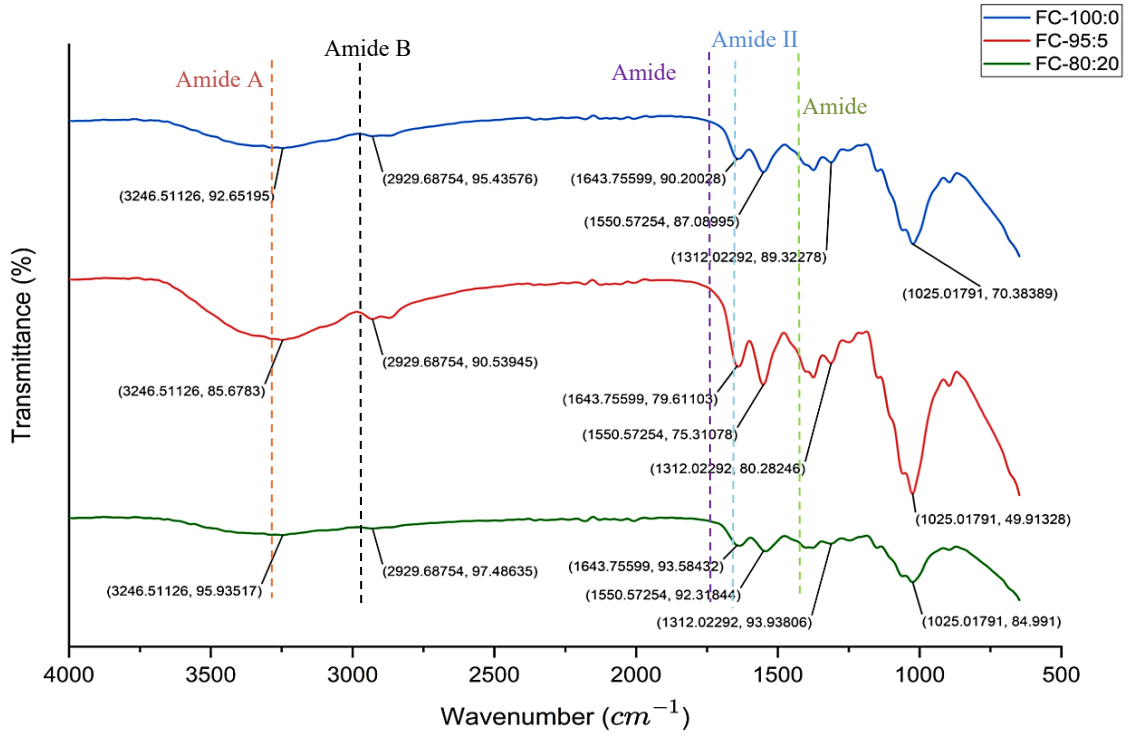


Figure 2: FTIR of chitosan film (FC-100:0), chitosan/gelatin film (FC-95:5) and chitosan/gelatin film (FC-80:20)

A band appearing at 1312 cm^{-1} (Amide III, C–N stretching with N–H deformation, transmittance 89.32 %) is a standard characteristic of the peptide backbone in protein-containing materials, while the sharp peak at 1025 cm^{-1} (transmittance 70.38 %) is the stretching vibrations of –C–O–C– bending [15]. When fish gelatin is added into the chitosan matrix (FC-95:5 and FC-80:20) several noticeable spectral changes occur and reflecting increased protein content and molecular interactions between both biopolymers. For FC-95:5, a slight narrowing of the Amide A band (~3246 cm^{-1}) was observed alongside a reduction in transmittance to 85.67 %, indicating stronger infrared absorption relative to FC-100:0 (92.65 %). This is attributed to localised molecular interactions introduced by gelatin at low concentration, where additional amine and hydroxyl groups enhance hydrogen bonding within the chitosan network [10]. In contrast, FC-80:20 showed increased

transmittance at the same band (95.93 %), indicating weaker absorption despite containing the highest gelatin content. The Amide B band confirms this non-monotonic behaviour.

FC-95:5 (90.53 %) shows stronger absorption than both FC-100:0 (95.43 %) and FC-80:20 (97.48 %), which is physically consistent with the protein content of each formulation. These opposing spectral responses between FC-95:5 and FC-80:20 indicate that the relationship between gelatin content and molecular interaction is non-linear, tentatively attributed to differences in film density and matrix uniformity at higher gelatin concentrations [16]. Since gelatin also exhibits an amide C=O band in the same region as the imine linkage (~1643 cm^{-1}), individual contributions of crosslinking and hydrogen bonding cannot be precisely separated by FTIR alone. The amide I and II functional groups also contribute to antibacterial activity, where the carbonyl (C=O) and N–H groups interact with bacterial cell walls to compromise their structure [14], though microbiological validation remains necessary. The consistent band near 1025 cm^{-1} confirms that the chitosan backbone remains stable after gelatin addition and crosslinking with glutaraldehyde. The complete transmittance and wavenumber data are summarised in Table 1.

Table 1: Functional Group for Each Peak of chitosan film (FC-100:0), chitosan/gelatin film (FC-95:5) and chitosan/gelatin film (FC-80:20)

Functional Group	Designation	Wavenumber (cm^{-1})	Transmittance (%)
Amide A (O–H and N–H stretching band)	FC-100:0	~3246	92.65
	FC-95:5		85.67
	FC-80:20		95.93
Amide B (Asymmetric stretching of CH, CH_2 groups and N–H contributions)	FC-100:0	~2929	95.43
	FC-95:5		90.53
	FC-80:20		97.48
Amide I (C=O stretching)	FC-100:0	~1643	90.20
	FC-95:5		79.61
	FC-80:20		93.58
Amide II (N–H bending and C–N stretching)	FC-100:0	~1550	87.08
	FC-95:5		75.31
	FC-80:20		92.31
Amide III (C–N stretching with N–H deformation)	FC-100:0	~1312	89.32
	FC-95:5		80.28
	FC-80:20		93.93
C-O-C stretching vibrations	FC-100:0	~1025	70.38
	FC-95:5		49.91
	FC-80:20		84.99

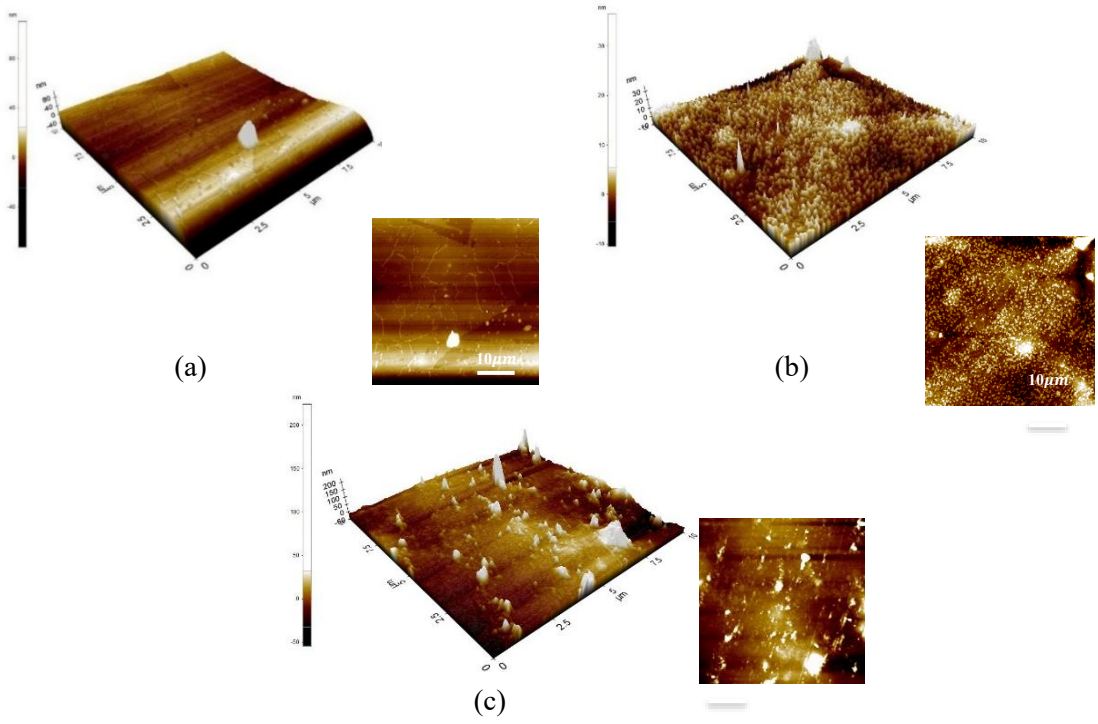
3.2 Atomic Force Microscope (AFM)

AFM analysis was conducted to investigate the surface topography and roughness parameters of the biofilms. Three conditions were studied which are (a) chitosan biofilm crosslinked with glutaraldehyde (FC-100:0), (b) chitosan/fish gelatin biofilm crosslinked with glutaraldehyde (FC-95:5) and (c) chitosan/fish gelatin biofilm crosslinked with glutaraldehyde (FC-80:20).The surface roughness was quantified using four parameters which are Roughness Peak to Valley (Rpv), Roughness Root Mean Square (Rq), Roughness Average (Ra), and Average Maximum Height of the Profile (Rz), are compared between the three samples. The results are summarized in Table 2 and illustrated in Figure 3. It was observed that FC-100:0 film exhibited moderate surface roughness values (Rpv = 133.547 nm, Rq = 7.978 nm, Ra = 5.054 nm). In contrast, the FC-95:5 film appeared smoother and more homogeneous, as shown in both 2D and 3D AFM images.

The introduction of fish gelatin reduced the roughness parameters to Rpv = 58.411 nm, Rq = 3.530 nm, and Ra = 2.479 nm, with a corresponding decrease in the average maximum height (Rz) from 130.739 nm for FC-100:0 to 53.778 nm for FC-95:5. While, the sample FC-80:20 the roughness parameters showed Rpv = 220.815 nm, Rq = 16.928 nm, Ra = 11.943 nm and the average maximum height Rz = 217.305 nm. This showed that when too much gelatin is added (20 %), the gelatin and chitosan do not mix evenly anymore. Instead, the gelatin may clump together or separate within the film, causing the surface to become uneven and rougher.

Table 2: Surface roughness parameter of chitosan film (FC-100:0), chitosan/gelatin film (FC-95:5) and chitosan/gelatin film (FC-80:20)

Sample	Surface Roughness Parameter			
	Roughness Peak to Valley, Rpv (nm)	Roughness Root Mean Square, Rq(nm)	Roughness Average, Ra(nm)	Average Maximum Height of the Profile, Rz(nm)
FC- 100:0	133.547	7.978	5.0535	130.739
FC- 95:5	58.411	3.530	2.479	53.778
FC- 80:20	220.815	16.928	11.943	217.305



(a) (b) (c)

Figure 3: 2D and 3D AFM Image of (a) chitosan film crosslinked with glutaraldehyde (FC-100:0), (b) chitosan/fish gelatin film crosslinked with glutaraldehyde (FC-95:5), and (c) chitosan/fish gelatin biofilm crosslinked with glutaraldehyde (FC-80:20)

The non-linear relationship between gelatin content and surface roughness, where FC-95:5 is smoother than FC-100:0, but FC-80:20 is considerably rougher, indicates that the optimal gelatin incorporation level for surface homogeneity lies closer to 5 % than 20 % under the fabrication conditions employed in this study. Surface topography is recognised as a factor that may influence bacterial adhesion behaviour at biomaterial surfaces [17]. In the literature, surface topography modifications on biopolymer-based materials have been associated with potential effects on cell

behaviour, including cell movement at the material interface [11]. However, the specific biological implications of the roughness values measured in this study, including any effects on cell adhesion, migration, or bacterial interaction cannot be determined from surface roughness data alone. Validation through dedicated in vitro cell adhesion, migration, and antibacterial assays is required before any biological conclusions can be drawn regarding the suitability of these biofilms for wound healing applications. The AFM data presented here are therefore interpreted strictly in terms of the physical surface characteristics of each biofilm formulation.

3.3 Contact Angle Measurement via Goniometer

The surface wettability of chitosan and fish gelatin film was evaluated using contact angle measurements to evaluate the hydrophilicity and hydrophobicity of the films. Table 3 shows the contact angle value for chitosan biofilm and chitosan /fish gelatin biofilm. The contact angle measurements presented in Table 3 and Figure 4 reveal the chitosan biofilm crosslinked with glutaraldehyde (FC-100:0), chitosan/fish gelatin biofilm crosslinked with glutaraldehyde (FC-95:5) and (c) chitosan/fish gelatin biofilm crosslinked with glutaraldehyde (FC-80:20) exhibit hydrophilic properties as each biofilm shows contact angles are below 90 °.

Table 3: Contact angle value for chitosan biofilm (FC-100:0), chitosan/fish gelatin biofilm (FC-95:5) and chitosan/fish gelatin biofilm (FC-80:20)

Resting Time	Contact Angle (°)			
	1st	2nd	3rd	Average
FC-100:0	74.70	74.50	76.70	75.3
FC-95:5	67.50	71.50	-	69.5
FC-80:20	63.30	67.10	67.20	65.9

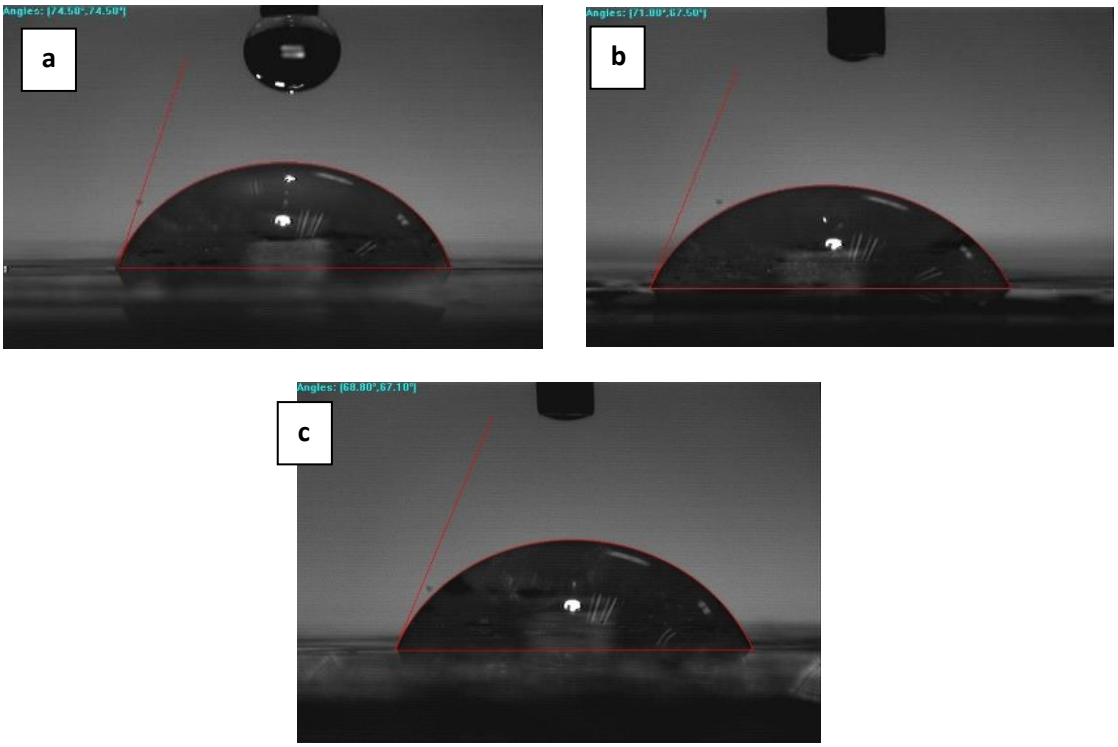


Figure 4: Contact angle of sample (a) chitosan biofilm (FC-100:0), (b) chitosan/fish gelatin biofilm (FC-95:5) and (c) chitosan/fish gelatin biofilm (FC-80:20)

The chitosan biofilm (FC-100:0) showed an average contact angle of 75.3 °, while the chitosan/fish gelatin biofilm (FC-95:5) and (FC-80:20) had a lower average contact angle of 69.5 ° and 65.9 °. These results suggest that all biofilms have good water absorption capabilities with the FC-100:0 being slightly less hydrophilic than the FC-95:5 and FC-80:20. The increased hydrophilicity of the chitosan/fish gelatin biofilm can be attributed to the presence of fish gelatin, which contains hydrophilic compounds such as amino acids and peptides, enhances its water interaction and wettability [18]. This showed that gelatin films derived from fish gelatin have many hydrophilic groups such as carboxyl and hydroxyl groups leading to high hydrophilicity in the materials [19]. These findings suggest that both materials from chitosan and gelatin are suitable for applications requiring water interaction such as wound dressing.

4. CONCLUSIONS

It can be concluded that the addition of fish gelatin to chitosan films improves their physiochemical characteristics and surface characteristics, making them suitable for wound healing applications. The combination of chitosan and fish gelatin shows great promise as a natural and biobased material for biomedical applications, particularly as a wound dressing. Moreover, this research provides detailed insights into the biofilm's structural and functional properties, such as:

- exhibits optimal surface wettability and uniform bioactive compound distribution
- satisfactory physicochemical properties that provide a foundation for further investigation into antimicrobial performance through future microbiological assays.

Additionally, it would be beneficial to evaluate the antibacterial properties of this biofilm in the future. Finally, it is suggested that chitosan/fish gelatin biofilm leads to an ideal material for biomedical coatings or protective layers as effective wound healing treatments.

Acknowledgements

This research was supported by Universiti Tun Hussein Onn Malaysia UTHM) through Multidisciplinary Research Grant, MDR (vot Q725) and Postgraduate Research Grant, GPPS (vot Q786).

Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

References

[1] Zhang, W., Hu, J., Wu, H., Lin, X. & Cai, L. (2025). Stimuli-responsive hydrogel dressing for wound healing. *APL Materials*, 13(1), 010601.

[2] Banerjee, D., Vydiam, K., Vangala, V. & Mukherjee, S. (2025). Advancement of nanomaterials- and biomaterials-based technologies for wound healing and tissue regenerative applications. *ACS Applied Bio Materials*, 8(3), 1877–1899.

[3] Hamdi, M., Sun, H., Pan, L., Wang, D., Sun, M., Zeng, Z., Li, S., Dong, Q. & Su, F. (2025). Chitosan and its derivatives as potential biomaterials for biomedical and pharmaceutical applications: A comprehensive review on green extraction approaches, recent progresses, and perspectives. *European Polymer Journal*, 229, 113882.

[4] Ndlovu, S. P., Ngece, K., Alven, S. & Aderibigbe, B. A. (2021). Gelatin-based hybrid scaffolds: promising wound dressings. *Polymers*, 13(17), 2959.

[5] Karydis-Messinis, A., Moschovas, D., Markou, M., Gkantzou, E., Vasileiadis, A., Tsirka, K., Gioti, C., Vasilopoulos, K. C., Bagli, E., Murphy, C., Salmas, C. E., Giannakas, A. E., Hatziloukas, E., Stamatis, H., Paipetis, A., Karakassides, M. A., Avgeropoulos, A. & Zafeiropoulos, N. E. (2023). Development, physicochemical characterization and in vitro evaluation of chitosan-fish gelatin-glycerol hydrogel membranes for wound treatment applications. C*arbohydrate Polymer Technologies and Applications*, 6, 100338.

[6] Peng, Q., Wen, F., Tang, X., Lu, S., Li, H., Wang, C., Chen, Z., Zhou, Z., Tan, S., Qin, H., Bi, Y., Wang, Z. & Kong, F. (2025). Carboxymethyl chitosan-gelatin based films filled with whey protein-stabilized nanoscale essential oil for skin wound healing: In vivo and in vitro studies. *International Journal of Biological Macromolecules*, 305(2), 141119.

[7] Hoang Thi, T. T., Do Thi, T. L. & Nguyen, D. H. (2022). Preparation and characterization of gelatin-based hydrogels encapsulating centella asiatica extract for biomedical applications. *Vietnam Journal of Science and Technology*, 60(3), 527–540.

[8] Al-Nimry, S., Dayah, A. A., Hasan, I. & Daghmash, R. (2021). Cosmetic, biomedical and pharmaceutical applications of fish gelatin/hydrolysates. *Marine Drugs*, 19(3), 145.

[9] D'souza, O. J., Pinto, J. P., Shettar, A. K., Narasagoudr, S. S., Masti, S. P. & Chougale, R. B. (2023). Biofunctionalization of chitosan/gelatin composite films reinforced Phyllanthus niruri extract for wound healing application. *Surfaces and Interfaces*, 43, 103567.

[10] Wegrzynowska-Drzymalska, K., Mlynarczyk, D. T., Chelminiak-Dudkiewicz, D., Kaczmarek, H., Goslinski, T. & Ziegler-Borowska, M. (2022). Chitosan-gelatin films cross-linked with dialdehyde cellulose nanocrystals as potential materials for wound dressings. *International Journal of Molecular Sciences*, 23(17), 9700.

[11] Cui, L., Yao, Y. & Yim, E. K. F. (2021). The effects of surface topography modification on hydrogel properties. *APL Bioengineering*, 5(3), 031509.

[12] Abdullah, Cai, J., Hafeez, M. A., Wang, Q., Farooq, S., Huang, Q., Tian, W. & Xiao, J. (2022). Biopolymer-based functional films for packaging applications: A review. *Frontiers in Nutrition*, 9, 1000116.

[13] Kim, D., Chan, I. N. M. D., Manalastas, T. M. S., Concepcion II, R. S., Sta. Agueda, J. R. H. & Bitangcor, R. K. B. (2025). Optimization of glutaraldehyde concentration in relation to swelling

behavior of PVA-PEG-BTB film using hybrid genetic metaheuristic algorithm. *Scientific Reports*, 15(1), 12582.

[14] Shahvalizadeh, R., Ahmadi, R., Davandeh, I., Pezeshki, A., Seyed Moslemi, S. A., Karimi, S., Rahimi, M., Hamishehkar, H. & Mohammadi, M. (2021). Antimicrobial bio-nanocomposite films based on gelatin, tragacanth, and zinc oxide nanoparticles – Microstructural, mechanical, thermo-physical, and barrier properties. *Food Chemistry*, 354, 129492.

[15] Khemkaew, P., Jaisan, C., Kingwascharapong, P., Rawdkuen, S., Karbowiak, T., Degraeve, P., Sai-Ut, S., Sangsawad, P., Kraithong, S. & Kaewprachu, P. (2025). Biobased antioxidant packaging from chitosan incorporating cashew leaf extract and TiO2 nanoparticles for soybean oil preservation. *LWT*, 228, 118053.

[16] Herrera-Vázquez, S. E., Dublán-García, O., Arizmendi-Cotero, D., Gómez-Oliván, L. M., Islas-Flores, H., Hernández-Navarro, M. D. & Ramírez-Durán, N. (2022). Optimization of the physical, optical and mechanical properties of composite edible films of gelatin, whey protein and chitosan. *Molecules*, 27(3), 869.

[17] Li, W., Thian, E. S., Wang, M., Wang, Z. & Ren, L. (2021). Surface design for antibacterial materials: From fundamentals to advanced strategies. *Advanced Science*, 8(19), 2100368.

[18] Pulla-Huillca, P. V., Gomes, A., Bittante, A. M. Q. B., A., Lourenço, V. R. & Sobral, P. J. d. A. (2021). Wettability of gelatin-based films: The effects of hydrophilic or hydrophobic plasticizers and nanoparticle loads. J*ournal of Food Engineering*, 297(2), 110480.

[19] Bustamante-Torres, M., Romero-Fierro, D., Arcentales-Vera, B., Palomino, K., Magaña, H. & Bucio, E. (2021). Hydrogels classification according to the physical or chemical interactions and as stimuli-sensitive materials. *Gels*, 7(4), 182.