

Original Article

Evaluation of Gelatin/Xanthan Gum based Biomaterial Enhanced with Barbaloin for Wound Healing Application: An *In Silico* and *In Vitro* Study

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In this study, a gelatin- and xanthan-gum-based biomaterial enhanced with barbaloin was developed and evaluated for its potential application in wound healing. The scaffold was designed to leverage the biocompatibility of gelatin, the structural stability provided by xanthan gum, and the therapeutic properties of barbaloin. Physicochemical characterization was conducted using FTIR, XRD, and SEM. XRD patterns indicated an amorphous structure, favorable for enhanced swelling and controlled drug release, whereas FTIR confirmed effective molecular interactions among the components. SEM imaging revealed a porous and interconnected architecture that is essential for cell migration and nutrient exchange. Swelling and porosity assessments demonstrated the ability of the scaffold to efficiently absorb wound exudates and maintain a moist environment conducive to tissue regeneration. Biocompatibility, evaluated via the MTT assay, showed high fibroblast viability, confirming the non-toxic nature of the material. Furthermore, molecular docking studies revealed strong binding affinities between barbaloin and wound healing-related proteins, suggesting its role in modulating inflammation and promoting tissue repair. Overall, the Gelatin/Xanthan Gum/Barbaloin scaffold exhibited excellent physicochemical characteristics, cytocompatibility, and therapeutic potential, making it a promising candidate for advanced wound dressing applications.

Introduction

In recent years, wound-healing research has shown significant advancements, focusing on improving the speed and quality of the healing process. Wound healing is divided into four phases: hemostasis, inflammation, proliferation, and remodelling [1]. Each phase is essential for the effective tissue and restoration of skin functionality [2]. The mechanism of wound healing is essential for developing advanced treatments, such as bioactive dressings, hydrogels, and tissue-engineered scaffolds, to support and accelerate the healing process [3]. Scaffolding is an essential component of tissue engineering [4]. Biomaterial scaffolds are engineered three-dimensional structures designed to mimic the natural Extracellular Matrix (ECM), which plays a crucial role in supporting proliferation, cell attachment, and differentiation during tissue regeneration. A perfect scaffold should be biocompatible, biodegradable, and bioactive [5].

Biomaterials are used to evaluate, treat, enhance, repair, and replace body tissues or organs [6]. The combination of Gelatin, Xanthan

Gum, and Barbaloin holds great promise for enhancing wound healing and regenerative medicine. Gelatin derived from collagen contains numerous functional groups and may be used in tissue scaffolds [7]. The high cell survival and lack of antigenicity of gelatin make it useful in medicines, wound dressings, and clinical adhesives. Selectivity and mass-producibility can be facilitated by form availability and cost-effectiveness [8]. Xanthan Gum (XG) is a microbial-derived high-molecular-weight heteropolysaccharide that is employed in the medical and tissue engineering fields because of its biocompatibility and gelling properties [9]. It is widely used in the pharmaceutical field as a thickener, suspender, and emulsifier as well as in the manufacture of biodegradable hydrogels for skin scaffolds [10].

Barbaloin is a phytochemical obtained from the leaves of Aloe vera plants. It enhances skin regeneration by promoting migration and cell proliferation, which are essential processes in wound healing [11]. It plays a crucial role in wound healing and is used as a skin repair material in tissue engineering. Therefore, the aim of this study was to fabricate a Gelatin/Xanthan Gum-based scaffold incorporating bar-

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baloin and to evaluate its physicochemical and biocompatibility properties. Furthermore, this study aimed to investigate its potential effectiveness as a wound-healing biomaterial.

Materials and Methods

Gelatin and Xanthan gum used for fabrication were purchased from SRL Chemicals (Sisco Research Laboratories). Barbaloin was procured from the Yucca Enterprises. The MTT and DMSO used for the cell proliferation study were obtained from SRL Chemicals.

In silico analysis

Network pharmacology

In network pharmacology, the PubChem database is a crucial resource used to access chemical structures (molecular formula, molecular weight, and canonical smiles), which were converted to the SDF format. Swiss ADME (<http://www.swissadme.ch/>) was used to predict and evaluate the pharmacokinetic properties (absorption, distribution, metabolism and excretion [ADME] of barbaloin. The canonical smiles are entered in the database and the violation (max 2 only) and bioavailability score (less than 1 or 1). Retrieving the data and downloading it in CSV format. The SuperPred web server links chemical similarities among drug-like compounds to molecular targets (<https://prediction.charite.de/>) [12]. It has been used to predict the target genes of the phytocompound (barbaloin). Gene cards (<https://www.genecards.org/>) are comprehensive online databases that provide detailed information on human genes, their functions, and related diseases. Potential therapeutic targets for barbaloin against wound healing were identified using an interactive Venn diagram. HUB genes were copied from the STRING Database (<https://string-db.org/>). The STRING database systematically collects and integrates protein-protein interactions, physical interactions, and functional associations [13]. The STRING interaction appears and downloads it in PNG format. The top 10 hub genes were identified to have a high degree of connectivity. Thus, these hub genes are potential therapeutic targets for wound healing.

Molecular docking

A molecular docking approach identifies amino acid interactions between the selected proteins and ligands that exhibit lower conformational energy. The top 10 drugs identified by virtual screening were subjected to molecular docking with AutoDock 4.2. The ligands were stored in a separate folder containing four major types of files. The folder should contain Autodock, Autogrid, two DAT files, the target protein in PDB format, and ligands in PDB format [14]. The prepared protein was docked with drugs obtained from virtual screening. The latest version of autodocking i.e. autodock 4 steps, involves the addition of non-polar hydrogen bonds. Kollman charge was added to the target proteins. Here, the ligand was subjected to flexible torsion, whereas the target protein was set to rigid. The docking process was based on the Lamarckian genetic algorithm. A grid box with the assigned parameters was generated by targeting the active sites of the target protein. After molecular docking was completed, the results were viewed using a DLG file. The files obtained from molecular docking interactions were stored for future purposes. The docked protein ligand complex was visualized using a BIOVIA Discovery Studio. They clearly depicted the amino acid residues of the target protein bound to the ligands. They provided both 2D and 3D struts of our complex in PNG format [15]. The interactions of all top 10 drugs were viewed using this approach.

Fabrication of biomaterials

Biomaterial fabrication was performed using a lyophilization technique. Gelatin (Gel, 5% w/v) was prepared in distilled water at 37°C, followed by the incorporation of 0.5% (w/v) XG under continuous stirring. The Gel/XG combination served as the control group, whereas the test groups were supplemented with 1 mM Barbaloin (Gel/XG/1 mM Barbaloin) and 10 mM Barbaloin (Gel/XG/10 mM Barbaloin). The prepared biomaterial suspensions were transferred to a 24-well plate and then frozen at -80°C for 72 h. Furthermore, they were freeze-dried to

obtain porous scaffolds [16,17]. Group I – Gel/XG; Group II – Gel/XG/1mM Barbaloin (lowest concentration of barbaloin); Group III – Gel/XG/10mM Barbaloin (highest concentration of barbaloin)

Physiochemical characterization

Fourier transform infrared spectroscopy (FTIR)

FTIR is an analytical technique that provides information about the functional groups present in the biomaterials. Approximately 50 mg of biomaterials from each group was weighed and analysed at wavelengths ranging from 4000 cm⁻¹ to 400 cm⁻¹ to determine the functional groups of the polymers blended into the biomaterial suspension [18].

X-ray diffraction (XRD)

XRD analysis is used to analyse the phase composition of biomaterials. Approximately 50 mg of the biomaterials from each group was placed onto the sample holder, which interacted with the X-rays, and Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) was usually generated and focused onto the sample. A detector records the intensity of diffracted X-rays between 20° and 90° (2 θ), generating diffraction patterns [19].

Scanning electron microscopy (SEM)

SEM is a microscopic technique that provides topographical and elemental compositional information about a biomaterial at the surface level. The porous structures of the biomaterials were analyzed at different magnifications from 1 μ m to 400 μ m [20].

Biochemical characterization

Porosity analysis

The porosity analysis of the composite scaffolds was performed using the liquid displacement method. This is the most commonly used technique for measuring the total porosity of porous biomaterials. Ethanol was used as the displacement liquid. The initial volume of liquid (ethanol) was recorded (V_{initial}). Equal weights of the biomaterials from each group were weighed and immersed in ethanol ($n=3$), and the total increase in liquid volume (V_{total}) was recorded. Following overnight incubation, the biomaterials were removed from the liquid, and the volume of the remaining liquid was recorded (V_{final}) [21]. The experiments were done in triplicates and the results were expressed as mean $\pm$ standard deviation. The porosity percentage (%) of the biomaterials was calculated using the following formula:

$$\text{Porosity (\%)} = \frac{V_{\text{initial}} - V_{\text{final}}}{V_{\text{total}} - V_{\text{final}}} \times 100$$

Swelling analysis

The biomaterials in each group were noted for their dry weight (W_o) ($n=3$). The scaffolds were immersed in 1x PBS solution at 37°C. The wet weights (W_i) of the scaffolds were recorded at 1, 2, and 3 h [22]. The experiments were done in triplicate, and the results were expressed as mean $\pm$ standard deviation. The swelling ratios of the biomaterials were calculated using the following formula:

$$\text{Swelling ratio} = \frac{W_i - W_o}{W_o}$$

In vitro degradation analysis

The dry weight of the scaffolds was recorded as W_o , followed by immersion in a 0.001% Lysozyme solution prepared in 1x PBS. The scaffolds were removed after the predetermined time intervals (7 days and 14 days) and freeze-dried to measure the dry weight of the degraded scaffolds (W_f). The experiments were done in triplicate, and the results were expressed as mean $\pm$ standard deviation. The degree of degradation (%) over weeks was determined using the following formula:

$$\text{Degree of degradation (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

Cell proliferation assessment

The MTT assay was performed to assess the proliferation rate of NIH-

3T3 fibroblasts. The conditioned media was prepared by treating the biomaterials with DMEM. The cells were grown in the 96 wells well-plate, treated with 125 µg/ml–1000µg/ml concentration of conditioned media at 37°C with 5% CO₂ for 24 h (n=3). Following incubation, the medium was discarded, and MTT (5 mg/ml w/v) was added and incubated for 2 h 30 min (37°C, 5% CO₂). 50 µl of DMSO was added to each well, and the wells were incubated in the dark for 15 min, followed by absorbance measurement at 570 nm [23]. The experiments were done in triplicate, and the results were expressed as mean ± standard deviation.

Results and Discussion

Network pharmacology analysis

The pharmacokinetic properties of the phytocompound were assessed using Swiss ADME. Barbaloin has passed Lipinski's rule of 5, and its bioavailability score is 0.55, indicating it is suitable for absorption into the systemic circulation. In Network pharmacology, the Venn diagram shows the intersection between barbaloin's molecular targets and wound-healing-related genes. Among the 105 known barbaloin targets, 72 overlapped with 6838 wound-healing genes. This overlap indicates that barbaloin can act through these 72 common targets to exert its therapeutic action on wound healing. A Venn diagram (figure 1A) revealed 72 overlapping genes shared between the barbaloin-predicted target and the wound healing-associated gene set. A Protein- Protein interaction (PPI) network was constructed using string database (figure 1B). The top 10

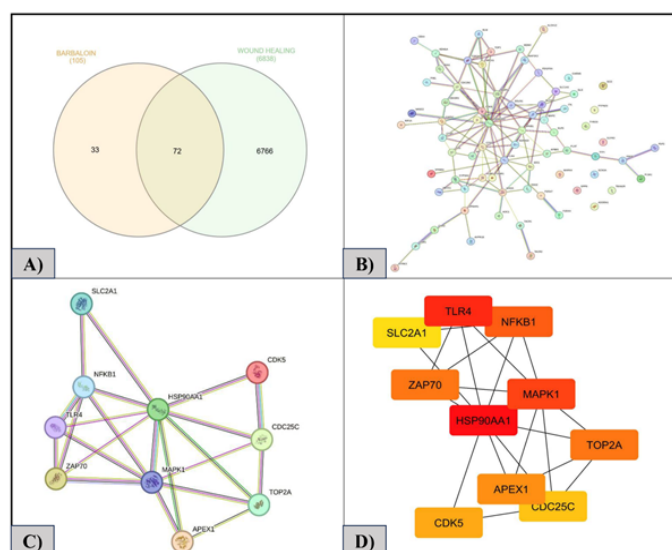


Figure 1: Barbaloin – Wound healing gene target 1A) Venn diagram of the Hub genes 1B) Network of protein-protein interaction 1C) The top 10 hub genes are analyzed through the STRING database 1D) The top 10 hub genes were ranked using the Cytoscape software

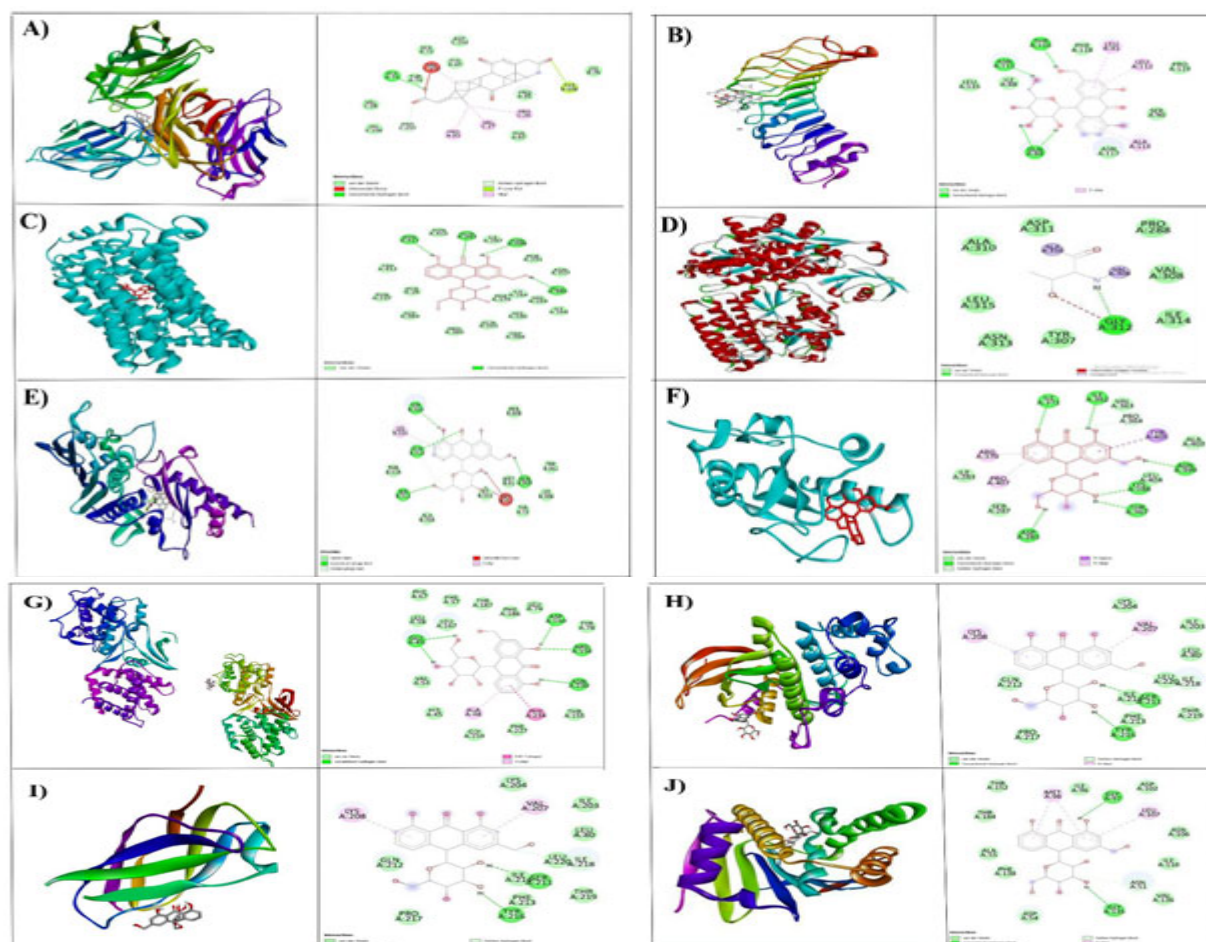


Figure 2: 3-Dimensional and 2-Dimensional map of the binding site between the target receptor and ligand (barbaloin); [1] APEX1 (-11.97 kcal/mol); [2] TLR4 (-9.23 kcal/mol); [3] SLC2A1 (-9.05 kcal/mol); [4] ZAP70 (-8.76 kcal/mol); [5] TOP2A (-8.54 kcal/mol); [6] CDC25C (-8.34kcal/mol); [7] CDK5 (-8.07 kcal/mol); [8] MAPK1 (-6.68 kcal/mol); [9] NFKB1 (-6.43kcal/mol); [10] HSP90AA1 (-5.96 kcal/mol)

Table 1: The binding affinities obtained as a result of molecular docking analysis conducted for barbaloin and the top 10 hub genes

Ligand	Receptor	PDB ID	Binding Affinity (kcal/mol)
Barbaloin	APEX1 – Apurinic/apyrimidinic endodeoxyribonuclease 1	5DFH	-11.97
	TLR4 – Toll-Like Receptor 4	2Z62	-9.23
	SLC2A1 – Solute Carrier Family 2-member 1	5EQI	-9.05
	ZAP70 – Zeta chain-associated Protein Kinase 70	5O76	-8.76
	TOP2A – Topoisomerase 2 alpha	1ZXM	-8.54
	CDC25C – Cell division cycle 25C	3OP3	-8.34
	CDK5 – Cycling dependent kinase 5	1UNL	-8.07
	MAPK1 – Mitogen-Activated Protein Kinase 1	6SLG	-6.68
	NFKB1 – Nuclear Factor Kappa B Subunit 1	1U36	-6.43
	HSP90AA1 – Heat Shock Protein 90 Alpha Family Class A Member 1	5NJX	-5.96

hub genes were analyzed using the STRING database (figure 1C). The top 10 hub genes were ranked using Cytoscape software (figure 1D). The top10 genes were APEX1, TLR4, SLC2A1, ZAP70, TOP2A, CDC25C, CDK5, MAPK1, NFKB1, and HSP90AA1. These genes are involved in various cellular processes, such as modulation of inflammation and tissue regeneration, which are essential components of wound healing.

APEX1 aids in repairing oxidative DNA lesions, thereby enabling cell proliferation and survival. It tends to activate transcription factors such as HIF-1 α , which play a major role in tissue regeneration [24]. TLR4, along with S100A9, upregulates the expression of Interleukin-1 β and TNF- α . Activation of TLR4 may contribute to the initial inflammatory response in pathological conditions involving wound repair [25]. SLC2A1, also known as GLUT1, enables the efficient uptake of glucose for various cellular activities, such as proliferation and migration, that occur during tissue repair [26].

Molecular docking analysis

Molecular docking was performed to analyse the binding interaction between barbaloin and the major target protein related to wound healing (figure 2). Of the top 10 receptors, APEX1 had the strongest binding energy with Barbaloin (-11.97 kcal/mol), as shown in table 1. This indicates that the binding is highly favourable and stable. TLR4 and

SLC2A1 Shown notable interaction with binding energies of -9.23 and -9.05 Kcal/mol respectively. CDC25C (-8.34 kcal/mol), ZAP70 (-8.76 kcal/mol), TOP2A (-8.54 kcal/mol) and CDK5 (-8.07 kcal/mol), which are essential for Cell regulation and tissue repair. MAPK1 (-6.68 kcal/mol), NFKB1 (-6.43 kcal/mol), and HSP90AA1 (-5.96 kcal/mol), indicating the role of barbaloin in the inflammatory signalling pathway.

APEX1, with a binding energy of -11.97 kcal/mol, plays an important role in the wound-healing process and is primarily involved in DNA repair, redox regulation, and inflammatory modulation. APEX1 is a biomarker for wound-healing disorders [27]. TLR4, with a binding energy -9.23 kcal/mol, plays an important role in the early inflammatory response in wound healing and managing chronic wounds. TLR4 can trigger the NFKB and MAPK signaling pathways, which lead to the production of pro-inflammatory cytokines [28]. SLC2A1, also known as GLUT1, is a glucose transporter protein with a binding energy -9.05 kcal/mol, and plays an essential role in wound healing by transporting glucose into cells. SLC2A1 facilitates cell proliferation, migration, and collagen production [29]. ZAP70, with a binding energy -8.76 kcal/mol, plays an important role in T cell receptor signaling. It may also help in wound healing, which coordinates tissue regeneration and inflammation [30]. TOP2A, with a binding energy -8.54 kcal/mol, plays a key role in the wound healing process by DNA segregation and DNA replication. It facilitates rapid cell turnover at the mid-stage of wound healing and serves as a biomarker for effective wound healing [31]. CDC25C and binding energy -8.34 kcal/mol, plays a major role in tissue regeneration, particularly in cell proliferation and migration [32]. This supports the proliferative stage of wound repair by stimulating cell division. CDK5 and its binding energy -8.07 kcal/mol play essential roles in the wound healing process by influencing cell migration and inflammatory signaling [33]. The β CDK5 gene is present in skin cells such as fibroblasts and keratinocytes, where it supports key repair mechanisms. MAPK1, binding energy -6.68 kcal/mol, plays a significant role in wound healing. It activates the ERK signaling pathway and promotes keratinocyte migration and fibroblast proliferation to fill wounds [34]. Nuclear Factor Kappa B Subunit 1 NFKB1, with a binding energy -6.43 kcal/mol, encodes a protein that plays a major role in the wound healing process [35]. In addition, it supports fibroblast activation and wound contraction through the TGF- β pathway. HSP90AA1 and its binding energy -5.96 kcal/mol play an essential role in the wound healing process. It supports the activation of signaling molecules, such as ERK and AKT, which are essential for supporting cell proliferation in the wounded region [36].

Fabrication of the biomaterials

The biomaterial suspension was prepared and frozen at -80°C for 72 h, followed by lyophilization to obtain porous biomaterial groups as shown in figure 3: Gel/XG, Gel/XG/1mM Barbaloin and Gel/XG/10mM Barbaloin.

**Figure 3: Top view and gross view of the fabricated scaffolds**

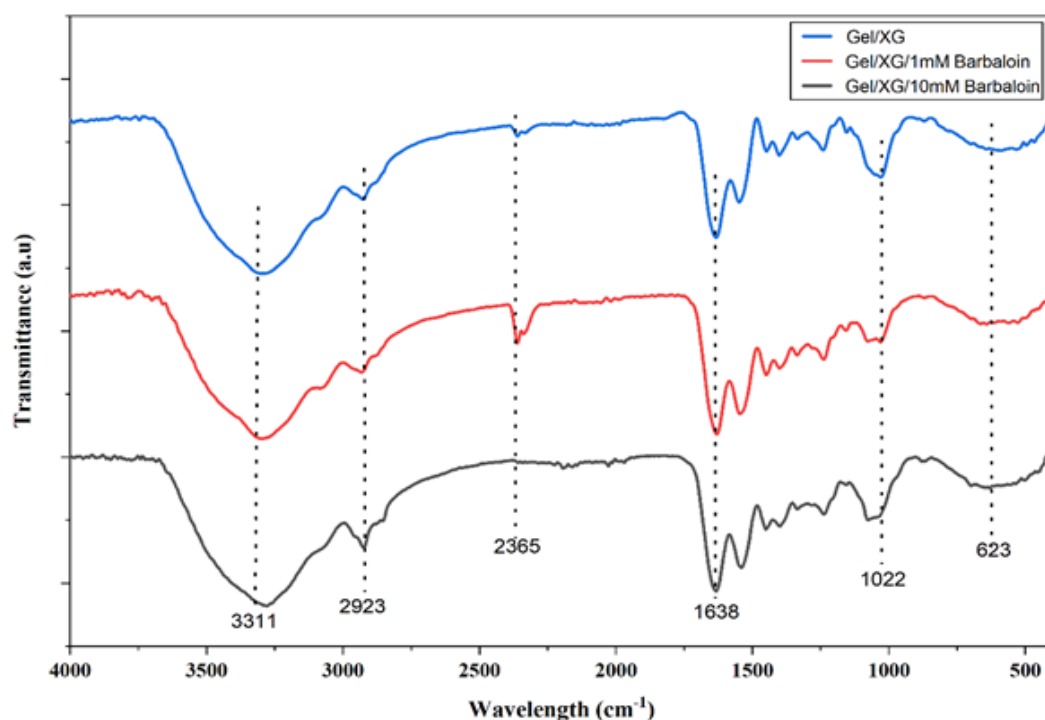


Figure 4: FTIR analysis of Gel/XG, Gel/XG/1mM Barbaloin and Gel/XG/10mM Barbaloin

Fourier transform infrared spectroscopy

The Gel/XG group exhibited an absorption band with a broad peak between 3300–3400 cm^{-1} which indicates O–H stretching of the hydroxyl group, and a peak at $\sim 1650 \text{ cm}^{-1}$ corresponding to C=O stretching of the gelatin [37]. More peaks in the range of 1000–1200 cm^{-1} corresponds to the C–O and C–O–C stretching vibrations of Xanthan Gum [38]. Upon the addition of 1 mM barbaloin (Gel/XG/1 mM barbaloin), there were marginal shifts and increased peak intensities in the O–H and amide I regions, indicating moderate hydrogen bond formation between barbaloin and the polymeric matrix. The spectrum obtained from this hydrogel still reflected the characteristic bands of the base hydrogel, confirming that the matrix structure persisted. In the case of the 10 mM Barbaloin group (Gel/XG/10 mM Barbaloin), there were much more pronounced shifts observed in the O–H $\sim 3300 \text{ cm}^{-1}$ and amide I $\sim 1650 \text{ cm}^{-1}$ surfaces and additional alterations were noted between the range of ~ 1000 – 1500 cm^{-1} . These findings indicate the successful incorporation of the polymers and alterations in the peaks created by the addition of barbaloin, as shown in figure 4.

X-ray diffraction analysis

The XRD patterns of all three formulations, Gel/XG, Gel/XG/1 mM Barbaloin and Gel/XG/10 mM Barbaloin exhibited broad diffuse halos without any sharp crystalline peaks, confirming their predominantly amorphous nature (figure 5A). The Gel/XG group showed a characteristic broad hump, indicating partial structural disorder due to intermolecular interactions between the gelatin and xanthan gum. Upon incorporation of barbaloin, the intensity of the amorphous halo further decreased with increasing concentration, demonstrating that barbaloin disrupted polymer chain packing and enhanced the amorphous characteristics of the biomaterial. The absence of distinct crystalline peaks of barbaloin confirmed its successful molecular dispersion within the polymer network [39]. This enhanced amorphous structure is advantageous for improving swelling behavior, biodegradability and sustained bioactive release in biomedical applications.

Scanning electron microscopy

In the SEM micrograph, the Gel/XG [(figure 5B – (i))] scaffold surface

was smoother and denser, with visible pores. The scaffold was slightly rough, indicating incomplete pore formation. Cell infiltration and nutrient distribution, which are essential for wound healing applications, may be limited by this heavy structure. Meanwhile Gel/XG/1mM Barbaloin [(figure 5B – (ii))] and Gel/XG/10mM Barbaloin [(figure 5B – (iii))] groups showed a more porous and connected structure in the SEM image. This porous nature is beneficial because it may improve proliferation, cell adhesion and tissue integration, indicating that the scaffold was influenced by the addition of barbaloin, supporting the formation of open structures that are excellent for wound healing applications.

Biochemical characterizations

Porosity analysis

The porosity graph (figure 6A) indicated that the Gel/XG formulation exhibited the highest porosity, demonstrating an interconnected and open structure that is favorable for facilitating cell attachment, nutrient diffusion, and tissue ingrowth [22]. With the incorporation of barbaloin, there was a gradual decline in porosity, with the Gel/XG/10mM Barbaloin group showing the lowest porosity among the other groups. This decrease can be attributed to the interaction of the barbaloin molecules with the polymer chains, leading to a denser and more compact network. While slightly reducing porosity, the incorporation of barbaloin may contribute to the improved mechanical integrity and controlled degradation properties of the scaffold.

Swelling analysis

Swelling analysis demonstrated that the Gel/XG group exhibited the highest swelling ratio across all time points, reflecting its hydrophilic nature and loosely crosslinked network capable of absorbing high amounts of fluid [40]. In contrast, the swelling capacity decreased in a concentration-dependent manner with the addition of barbaloin, particularly in the Gel/XG/10mM Barbaloin group (figure 6B). This reduction in swelling may suggest a higher crosslinking density and reduced free volume within the polymer matrix owing to stronger intermolecular interactions between barbaloin and the scaffold components. Controlled swelling is advantageous for maintaining scaffold stability

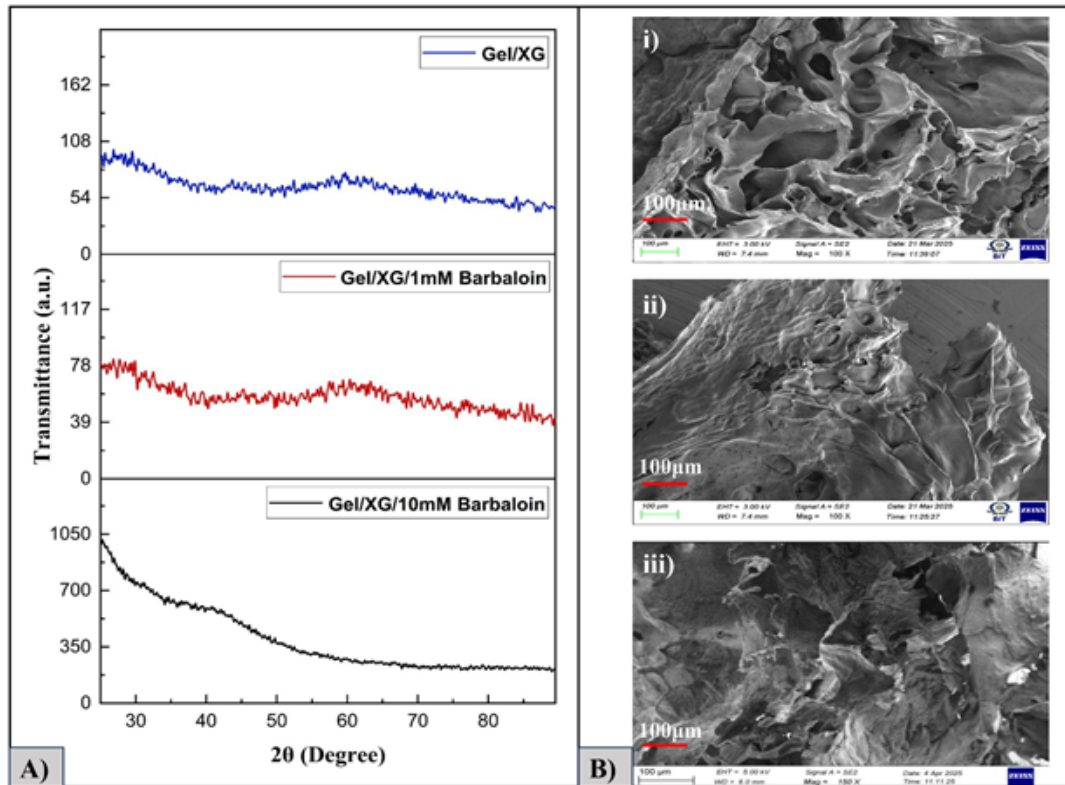


Figure 5 A) XRD spectrum of the biomaterials plotted using Origin software B) The microscopic images demonstrating the porous architecture of the biomaterials

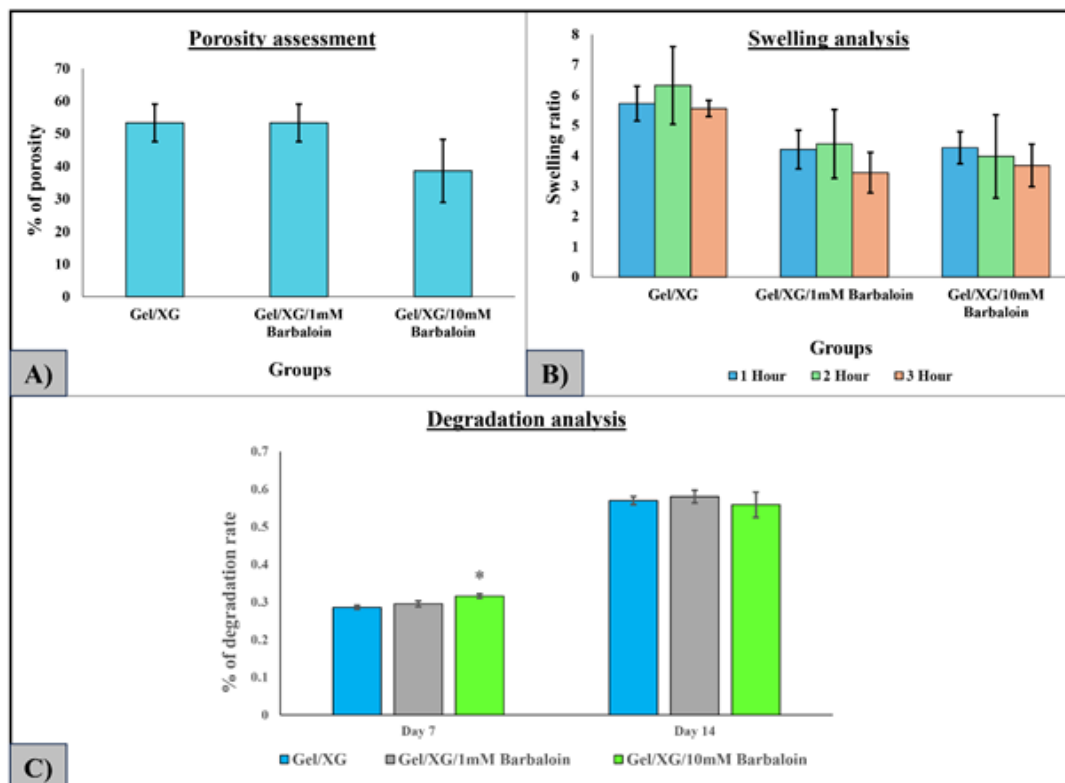


Figure 6: A) Porosity assessment of the biomaterial 7B) Swelling studies 7C) *In-vitro* degradation profile [Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance is indicated by * $p < 0.05$].

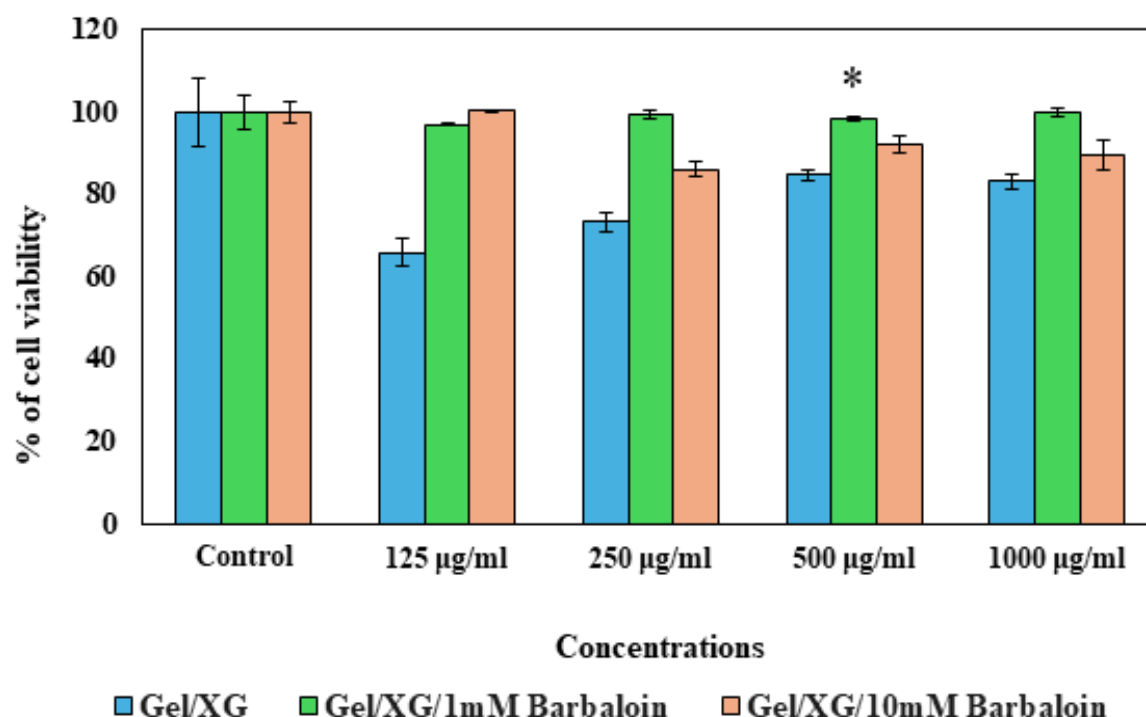


Figure 7: Cell proliferation analysis using MTT assay. The percentage of cell viability was determined to evaluate the cytocompatibility and proliferative potential of the scaffold-conditioned media [Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance is indicated by * $p < 0.05$]

and ensuring sustained release of bioactive molecules in biomedical applications.

In vitro degradation analysis

The *in vitro* degradation behavior of the scaffolds was evaluated over 7 and 14 days (figure 6C). All groups exhibited a gradual increase in degradation with increasing incubation time, indicating a time-dependent degradation pattern. On day 7, the Gel/XG/1 mM barbaloin and Gel/XG/10 mM barbaloin groups showed slightly higher degradation compared to the Gel/XG group. By day 14, all scaffold groups demonstrated a further increase in degradation. However, the differences among the groups were minimal, indicating a comparable degradation trend at the later stage. Overall, the scaffolds exhibited a controlled and progressive degradation profile, which is desirable for wound healing applications, as gradual scaffold degradation can facilitate fibroblast infiltration and extracellular matrix deposition by NIH3T3 cells, while maintaining structural support during the early stages of tissue repair [41].

In vitro cell-proliferation assessment

The MTT assay results demonstrated that all three biomaterial groups, Gel/XG, Gel/XG/1mM Barbaloin and Gel/XG/10mM Barbaloin exhibited excellent cytocompatibility with NIH3T3 fibroblast cells after 24 h of incubation (figure 7). At lower concentrations (125 and 250 µg/mL), the barbaloin-loaded scaffolds showed slightly higher cell viability than the pure Gel/XG group, indicating that the presence of barbaloin enhanced cell proliferation due to its bioactive and antioxidant properties. Even at higher concentrations (500 and 1000 µg/mL), all the groups had maintained cell viability above 70%, confirming the non-toxic and biocompatible nature of the scaffolds. Notably, the Gel/XG/1mM Barbaloin group consistently promoted greater cell proliferation, suggesting that this concentration may provide the optimal microenvironment for fibroblast attachment and metabolic activity. Overall, the results confirm the suitability of the developed biomaterials for wound healing applications because of their ability to support NIH3T3 cell growth.

Conclusion

The present study successfully fabricated a Gelatin/Xanthan Gum-based scaffold incorporated with barbaloin and demonstrated its suitability for wound healing applications through systematic *in-silico*, physicochemical, biochemical and biological evaluations. *In-silico* network pharmacology and molecular docking confirmed that barbaloin strongly interacts with key wound-healing-related target proteins such as APEX1, TLR4 and SLC2A1, indicating its therapeutic role in modulating inflammation and promoting tissue regeneration at the molecular level. FTIR confirmed strong intermolecular interactions between the polymers and barbaloin, while XRD revealed an amorphous structure favorable for controlled bioactive release and enhanced swelling. SEM analysis showed a well-defined porous and interconnected morphology in the barbaloin-loaded scaffolds, supporting cell infiltration and nutrient diffusion. Porosity and swelling studies demonstrated that the incorporation of barbaloin slightly reduced porosity and swelling compared to the control, which is indicative of improved structural stability and sustained-release behaviour. The MTT assay results confirmed excellent cytocompatibility with enhanced fibroblast viability, particularly at lower concentrations of barbaloin, highlighting its role in promoting cell proliferation and tissue regeneration. Overall, these findings confirm that the developed scaffold not only provides a structural matrix but also actively contributes to biological healing through its bioactive components.

However, the study has certain limitations. Antioxidant validation of the scaffold and fibroblast migration assays was not performed and molecular confirmation of the proposed redox-regulatory mechanism (APEX1 gene expression) requires further investigation. Future perspectives include optimizing the barbaloin dosage for clinical efficacy, validating *in vivo* wound closure rates, assessing long-term biodegradation behavior, expanding its application to chronic wound models and developing its translational potential for commercial wound care therapies.

References

- Boyce ST, Lalley AL. Tissue engineering of skin and regenerative medicine for wound care. *Burn Trauma*. 6, 4 (2018).
- Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. *Pharmaceutics*. 12(8) (2020).
- Martin P. Wound healing—aiming for perfect skin regeneration. *Science*. 276(5309), 75–81 (1997).
- Chan BP, Leong KW. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *Eur Spine J*. 17(Suppl 4), 467–479 (2008).
- Kammona O, Tsanakisidou E, Kiparissides C. Recent developments in 3D-(bio)printed hydrogels as wound dressings. *Gels*. 10(2) (2024).
- Chattopadhyay S, Raines RT. Collagen-based biomaterials for wound healing. *Biopolymers*. 101(8), 821–833 (2014).
- Tanaka A, Nagate T, Matsuda H. Acceleration of wound healing by gelatin film dressings with epidermal growth factor. *J Vet Med Sci*. 67(9), 909–913 (2005).
- Zheng Y, et al. Gelatin-based hydrogels blended with gellan as an injectable wound dressing. *ACS Omega*. 3(5), 4766–4775 (2018).
- Piola B, Sabbatini M, Gino S, Invernizzi M, Renò F. 3D bioprinting of gelatin–xanthan gum composite hydrogels for growth of human skin cells. *Int J Mol Sci*. 23(1) (2022).
- Alves A, et al. Xanthan gum–konjac glucomannan blend hydrogel for wound healing. *Polymers*. 12(1) (2020).
- Singh N, Goyal K, Sondhi S, Jindal S. Traditional and medicinal use of barbaloin: potential for the management of various diseases. *J Appl Pharm Res*. 8(3), 21–30 (2020).
- Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res*. 42(W32–W38) (2014).
- Li X, Wei S, Niu S, Ma X, Li H, Jing M, Zhao Y. Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. *Comput Biol Med*. 144, 105389 (2022).
- Zhang Y, Li Z, Wei J, Kong L, Song M, Zhang Y, Xiao X, Cao H, Jin Y. Network pharmacology and molecular docking reveal the mechanism of Angelica dahurica against osteosarcoma. *Medicine (Baltimore)*. 101(44), e31055 (2022).
- Zhang X, Zhu X, Gu W, Li X, Niu T, Mao P, Yuan H. Elucidating the mechanism of phthalates induced osteoporosis through network toxicology and molecular docking. *Ecotoxicol Environ Saf*. 291, 117820 (2025).
- Kumar S, Lahiri C, Chaudhary S, Paul P, Verma YK. Design, development and characterisation of an optimised scaffold to enhance cell proliferation for tissue repair. *J Microencapsul*. 40(2), 82–97 (2023).
- Amini N, Hivechi A, Asadpour S, et al. Fabrication and characterization of bilayer scaffolds made of decellularized dermis/nanofibrous collagen for healing of full-thickness wounds. *Drug Deliv Transl Res*. 13(6), 1766–1779 (2023).
- Riaz T, Zeeshan R, Zarif F, et al. FTIR analysis of natural and synthetic collagen. *Appl Spectrosc Rev*. 53 (2018).
- Sharma S, Madhyastha H, Swetha KL, et al. Development of an in-situ forming, self-healing scaffold for dermal wound healing: in-vitro and in-vivo studies. *Mater Sci Eng C*. 128, 112263 (2021).
- Agocsova V, Culenova M, Birova I, et al. Resorbable biomaterials used for 3D scaffolds in tissue engineering: a review. *Materials*. 16(12) (2023).
- Tihăuan BM, Pircalabioru GG, Bucos MA, et al. Crosslinked collagenic scaffold behavior evaluation by physico-chemical, mechanical and biological assessments in an in vitro microenvironment. *Polymers*. 14(12) (2022).
- Soleimanpour M, Mirhaji SS, Jafari S, et al. Designing a new alginate–fibrinogen biomaterial composite hydrogel for wound healing. *Sci Rep*. 12(1), 7213 (2022).
- Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc*. 2018(6) (2018).
- Wang L, Chen R, Zhang Y. miR-296-3p targets APEX1 to suppress cell migration and invasion of non-small-cell lung cancer. *Oncol Lett*. 18(3), 2612–2618 (2019).
- Davis FM, Kimball A, denDekker A, et al. Histone methylation directs myeloid TLR4 expression and regulates wound healing following cutaneous tissue injury. *J Immunol*. 202(6), 1777–1785 (2019).
- Mo Y, Wang Y, Zhang S, et al. Circular RNA circRNF13 inhibits proliferation and metastasis of nasopharyngeal carcinoma via SUMO2. *Mol Cancer*. 20(1), 112 (2021).
- Li M, Yang X, Lu X, et al. APE1 deficiency promotes cellular senescence and premature aging features. *Nucleic Acids Res*. 46(11), 5664–5677 (2018).
- Chen L, Guo S, Ranzer MJ, DiPietro LA. Toll-like receptor 4 has an essential role in early skin wound healing. *J Invest Dermatol*. 133(1), 258–267 (2013).
- Takahashi H, Kaminski AE, Zieske JD. Glucose transporter 1 expression is enhanced during corneal epithelial wound repair. *Exp Eye Res*. 63(6), 649–659 (1996).
- Wang H, Kadlecck TA, Au-Yeung BB, et al. ZAP-70: an essential kinase in T-cell signaling. *Cold Spring Harb Perspect Biol*. 2(5), a002279 (2010).
- Yang Y, Liu S, Xiao X. TOP2A promotes proliferation, migration, and inflammatory response in M5-treated keratinocytes by binding CTBP1 to activate Wnt/ β -catenin signaling. *Cell Biochem Biophys*. (2024).
- Liu K, Zheng M, Lu R, et al. The role of CDC25C in cell cycle regulation and clinical cancer therapy: a systematic review. *Cancer Cell Int*. 20(1), 213 (2020).
- Xu S, Li X, Gong Z, et al. Proteomic analysis of the human cyclin-dependent kinase family reveals a novel CDK5 complex involved in cell growth and migration. *Mol Cell Proteomics*. 13(11), 2986–3000 (2014).
- Deng M, Chen WL, Takatori A, et al. A role for the mitogen-activated protein kinase kinase 1 in epithelial wound healing. *Mol Biol Cell*. 17(8), 3446–3455 (2006).
- Chen J, Chen Y, Chen Y, et al. Epidermal CFTR suppresses MAPK/NF- κ B to promote cutaneous wound healing. *Cell Physiol Biochem*. 39(6), 2262–2274 (2016).
- Wiegand C, Hipler UC, Elsner P, Tittelbach J. Keratinocyte and fibroblast wound healing in vitro is repressed by non-optimal conditions but the reparative potential can be improved by water-filtered infrared A. *Biomedicines*. 9(12) (2021).
- Raja IS, Fathima NN. A gelatin-based antioxidant enriched biomaterial by grafting and saturation: towards sustained drug delivery from antioxidant matrix. *Colloids Surf B Biointerfaces*. 128, 537–543 (2015).
- Jaberifard F, Almajidi YQ, Arsalani N, Ghorbani M. A self-healing crosslinked-xanthan gum/soy protein-based film containing halloysite nanotube and propolis with antibacterial and antioxidant activity for wound healing. *Int J Pharm*. 10(656), 124073, (2024).
- Borse S, Nangare S, Bafna P, Jain P, Zavar L. Barbaloin loaded chitosan-gum kondagogu polyelectrolyte complex based biocomposites film for enhanced antibacterial, antioxidant and wound healing activity. *Surfaces and Interfaces*. 56, 105506, (2025).
- Balasubramanian R, Kim SS, Lee J. Novel synergistic transparent k-Carrageenan/Xanthan gum/Gellan gum hydrogel film: Mechanical, thermal and water barrier properties. *Int J Biol Macromol*. 118(Pt A), 561–568, (2018).
- Negut I, Dorcioman G, Grumezescu V. Scaffolds for Wound Healing Applications. *Polymers (Basel)*. 12(9), 2010, (2020).