RESEARCH PAPER

"Design and Molecular Docking-Guided Mechanistic Insights of Stevioside loaded Pluronic F127–Agar Hydrogel for Skin Tissue Regeneration"

Sai Krishna Bhimavarapu[1], Shanmugarajan T.S[2*]

[1]Research Scholar, Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS). PV Vaithiyalingam Rd, Velan Nagar, Krishnapuram, Pallavaram, Chennai, Tamil Nadu 600117.

[2*]Professor, Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS). PV Vaithiyalingam Rd, Velan Nagar, Krishnapuram, Pallavaram, Chennai, Tamil Nadu 600117.

Abstract

This study developed and mechanistically evaluated a stevioside-loaded Pluronic F127–agar hydrogel for skin tissue regeneration. Agar concentration was varied to optimize gelation behavior, structural integrity, and drug delivery performance. Increasing agar concentration reduced the sol–gel transition temperature (34.8 ± 0.6°C to 28.6 ± 0.6°C) and gelation time (142 ± 6 s to 52 ± 4 s), while increasing gel fraction (61.4 ± 2.1% to 96.4 ± 0.9%), indicating enhanced network formation. Molecular docking revealed strong binding affinities of stevioside toward VEGF-A (−8.13 kcal/mol) and MMP-9 (−8.55 kcal/mol), with moderate interaction toward TGF-β1 and IL-1β. The optimized hydrogel exhibited favorable gelation, stability, and sustained release characteristics, highlighting its potential as a localized drug delivery platform for skin regeneration and wound healing.

Keywords: Hydrogel; Pluronic F127; Agar; Stevioside; Molecular docking; VEGF-A; MMP-9; Skin regeneration; Controlled drug delivery

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1. Introduction

The tourism industry has undergone a substantial transformation over the past two decades, shifting from conventional Skin tissue regeneration is a complex and highly coordinated biological process involving inflammation, angiogenesis, extracellular matrix remodeling, and cellular proliferation. Successful wound healing requires not only appropriate biological signaling but also a favorable local microenvironment that supports tissue repair and regeneration. However, conventional wound management strategies often fail to provide sustained drug availability and optimal wound conditions, particularly in chronic wounds, resulting in delayed healing and impaired tissue recovery. In recent years, hydrogel-based systems have gained considerable attention as advanced wound dressings owing to their ability to maintain a moist environment, facilitate gas exchange, support cellular activity, and enable localized drug delivery.1

Among various hydrogel systems, Pluronic F127 has emerged as a promising thermoresponsive polymer due to its reversible sol-to-gel transition near physiological temperature and in situ gel formation. Nevertheless, Pluronic F127 alone exhibits limitations such as poor mechanical strength, rapid erosion, and limited structural stability, which can compromise sustained drug delivery. To address these drawbacks, natural polymers such as agar have been incorporated to improve gel strength, network integrity, and water-retention capacity.2

Stevioside, a naturally occurring diterpene glycoside obtained from Stevia rebaudiana, possesses anti-inflammatory, antioxidant, and tissue-regenerative properties that make it a promising candidate for wound-healing applications. However, its therapeutic effectiveness is restricted by rapid diffusion and inadequate retention at the target site. Incorporation into a hydrogel-based delivery system may overcome these limitations by providing controlled and sustained release.3

Furthermore, molecular docking studies with key wound-healing proteins, including VEGF-A, TGF-β1, MMP-9, and IL-1β, can provide mechanistic insight into the biological relevance of Stevioside. Therefore, the present study aimed to develop and optimize a stevioside-loaded Pluronic F127–agar hydrogel and evaluate its physicochemical characteristics and molecular interactions relevant to skin tissue regeneration.

*Author for Correspondence: smrajan.sps@vistas.ac.in

2.METHODOLOGY

2.1 Sol–Gel Transition

Temperature To measure the thermoresponsive gelation behaviour of the Pluronic F127-based hydrogel system, the sol gel transition temperature of each formulation was measured. A volume of hydrogel was put into a glass vial that was slowly heated in a temperature-controlled water bath under constant observation. The vial was inverted at every step of incremental temperature to determine the behavior of the flow. The temperature at which the hydrogel stopped flowing was noted to be the gelation temperature. This is an essential parameter in Pluronic-based wound-healing systems since the formulation must be flowable at refrigerated or room-temperature conditions to be administered yet form a stable gel at skin or body temperature after application.4

2.2 Gelation Time

Gelation time was measured as the time taken by the formulation to change its flowable sol state to a non-flowing gel state when subjected to physiological or skin-relevant temperature. A predetermined amount of hydrogel at 4°C was placed in a vial and incubated at 32-37C which was skin and near-body conditions. A stopwatch was initiated as soon as possible and the vial inversion technique was employed to identify the point where no flowing could be seen. Gelation time was timed in the form of seconds. This parameter is very important since wound-healing hydrogels are not to be left too liquid after their administration, and it is necessary that they should provide ample time to place the hydrogel uniformly and cover defects before they gel.5

2.3 Sol–Gel Fraction Analysis

Sol–gel fraction analysis was carried out to determine the structural integrity and effective network formation of the hydrogel. Hydrogel was dried to constant weight and the weight was recorded as W0. The dried hydrogel was then placed in distilled water and allowed to extract the soluble polymer part after 24 hours. 6 The remaining insoluble gel portion was re-dried and the weight taken as W1. The equations used were:

Gel Fraction (%) = (W1 / W0) × 100 Sol Fraction (%) = 100 − Gel Fraction

Large gel fraction means that the polymer is forming a better network and has high structural stability, which is crucial to the long-term retention of the hydrogel in the wound location.6

2.4 Molecular Docking Analysis

Molecular docking studies were performed to evaluate the interaction of Stevioside with key skin regeneration-related proteins, including VEGF-A, MMP-9, IL-1β, and TGF-β1. Protein structures were retrieved from the RCSB Protein Data Bank, pre-processed, and energy-minimized prior to docking. The Stevioside structure was obtained from PubChem, optimized using the MMFF94 force field, and prepared for docking. Simulations were conducted using the SwissDock server with the EADock DSS algorithm. Binding affinities were assessed using estimated free energy (ΔG), cluster ranking, and interaction stability. Docking results were visualized using PyMOL and Discovery Studio Visualizer to identify key interactions and evaluate the potential role of Stevioside in angiogenesis, inflammation regulation, extracellular matrix remodeling, and tissue regeneration. .7-9

3.RESULTS AND DISCUSSION

3.1 Sol–Gel Transition Temperature

Increasing agar concentration reduced the sol–gel transition temperature from 34.8 ± 0.6°C (F1) to 28.6 ± 0.6°C (F8), indicating enhanced network stabilization and earlier gel formation. This behavior is advantageous for wound-healing applications, where hydrogels should remain flowable during administration and rapidly form gels at physiological temperatures. Formulations F4–F6 exhibited the most desirable transition temperatures, providing an optimal balance between handling convenience and reliable in situ gelation.

3.2 Gelation Time

Gelation time decreased progressively from 142 ± 6 s (F1) to 52 ± 4 s (F8) with increasing agar concentration, reflecting enhanced network formation. Faster gelation improves retention at the wound site; however, excessively rapid gelation may hinder uniform application. Among all formulations, F5 exhibited an optimal gelation time of 79 ± 3 s, providing a favorable balance between ease of administration and effective site-specific gel formation.

3.3 Sol–Gel Fraction Analysis

Gel fraction increased from 61.4 ± 2.1% (F1) to 96.4 ± 0.9% (F8), while the sol fraction decreased correspondingly, indicating improved polymer incorporation and network integrity with increasing agar concentration. A higher gel fraction reflects enhanced structural stability and resistance to dissolution, which are desirable for wound-healing applications. Although F8 showed the highest gel fraction, formulations F5 and F6 provided a more balanced combination of network strength, handling characteristics, and functional performance.

Table1 Physico chemical characterisation of the of the developed formulations

Formulation	Temperature (°C ± SD)	Gelation Time (sec ± SD)	Gel Fraction (%) ± SD	Sol Fraction (%)
F1	34.8 ± 0.6	142 ± 6	61.4 ± 2.1	38.6
F2	33.2 ± 0.5	126 ± 5	68.2 ± 1.8	31.8
F3	31.6 ± 0.4	108 ± 4	74.6 ± 1.6	25.4
F4	30.8 ± 0.5	92 ± 3	81.3 ± 1.5	18.7
F5	30.4 ± 0.3	79 ± 3	87.9 ± 1.3	12.1

F6	29.9 ± 0.4	70 ± 4	91.2 ± 1.2	8.8
F7	29.2 ± 0.5	61 ± 5	94.1 ± 1.0	5.9
F8	28.6 ± 0.6	52 ± 4	96.4 ± 0.9	3.6

3.4 Interaction with Vascular Endothelial Growth Factor A (VEGF-A)

3.4.1 Stevioside

Molecular docking analysis of stevioside with Vascular Endothelial Growth Factor A (VEGF-A, PDB ID: 3QTK) using the SwissDock server identified a favorable binding conformation in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −8.13 kcal/mol and an AC score of 231.39, indicating a strong and thermodynamically stable interaction. Binding interaction analysis revealed that stevioside forms multiple hydrogen bonds through its hydroxyl and glycosidic groups, along with hydrophobic and van der Waals interactions within the VEGF-A binding cavity, contributing to the stability of the ligand–protein complex. The strong binding affinity suggests that stevioside may modulate VEGF-A-mediated angiogenic pathways involved in wound healing and skin regeneration. Such interactions could promote neovascularization, endothelial cell proliferation, and improved tissue vascularization, thereby enhancing granulation tissue formation, accelerating re-epithelialization, and supporting overall tissue repair and regeneration.

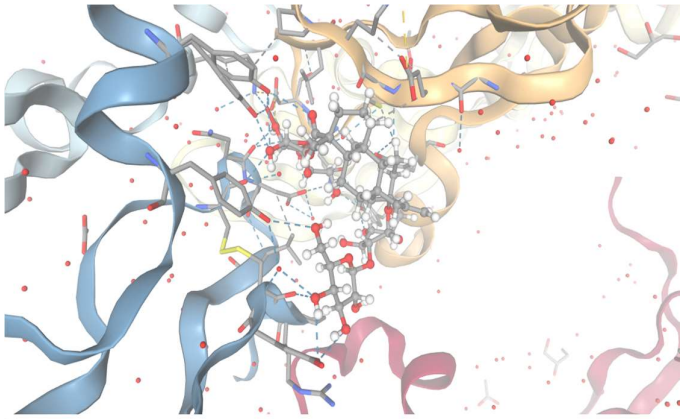

Figure 1. VEGF-A Protein Docking study of the Stevioside molecule

3.4.2 Pluronic F127/ Agar Hydrogel

Molecular docking analysis of the Pluronic F127/Agar hydrogel-associated ligand system with Vascular Endothelial Growth Factor A (VEGF-A, PDB ID: 3QTK) was performed using the SwissDock server with the Attracting Cavities 2.0 algorithm. The most favorable binding pose was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.69 kcal/mol and an AC score of 79.50, indicating a moderate and thermodynamically favorable interaction. The clustering of conformations around similar energy values suggested a consistent binding pattern, while buried cavity prioritization indicated preferential occupation of energetically favorable regions within the protein structure. Interaction analysis revealed the presence of non-covalent interactions, primarily hydrogen bonding, polar interactions involving hydroxyl and ether groups, and weak hydrophobic contacts associated with the polymer backbone, contributing to stabilization of the ligand within the binding cavity. The observed binding affinity suggests that the hydrogel components possess moderate interaction capability with VEGF-A, a key regulator of angiogenesis and wound healing. However, the hydrogel is intended primarily as a drug delivery matrix rather than a bioactive ligand, and the docking results mainly reflect the limited direct biological activity of the polymeric components while supporting their potential compatibility with skin regeneration applications.

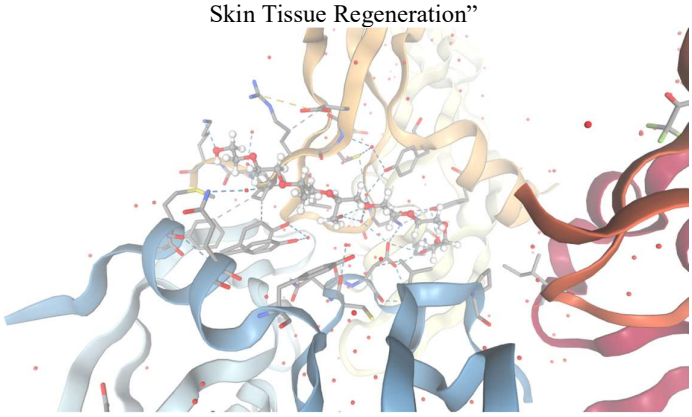

Figure 2. VEGF-A Protein Docking study of the Pluronic F127/Agar hydrogel

3.4.3 Stevioside Loaded Hydrogel

Molecular docking analysis of the stevioside-loaded Pluronic F127/Agar hydrogel system with Vascular Endothelial Growth Factor A (VEGF-A, PDB ID: 3QTK) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.43 kcal/mol and an AC score of 99.14, indicating a thermodynamically favorable but moderate interaction with the target protein. The clustering of conformations around similar energy values suggested a consistent binding pattern, while cavity prioritization indicated preferential occupation of energetically favorable regions within the VEGF-A structure. Interaction analysis revealed the involvement of ARG98 (chain B) and the presence of general non-covalent interactions, including hydrogen bonding, polar interactions arising from hydroxyl and ether groups, and hydrophobic contacts contributed by the composite ligand system. The observed binding affinity suggests that the stevioside-loaded hydrogel possesses moderate interaction capability with VEGF-A, a key regulator of angiogenesis and wound healing. Although the interaction strength was lower than that typically observed for free small-molecule ligands, this is expected due to the complex polymeric nature of the hydrogel representation. Importantly, the therapeutic role of the hydrogel is primarily associated with the controlled and sustained release of stevioside rather than direct protein binding. The interaction with VEGF-A supports the potential biological relevance of the released drug, while the hydrogel matrix provides prolonged retention and localized delivery. Collectively, these findings suggest that the stevioside-loaded Pluronic F127/Agar hydrogel may support wound healing and skin regeneration through a combination of sustained drug release and favorable interaction with angiogenesis-related pathways.

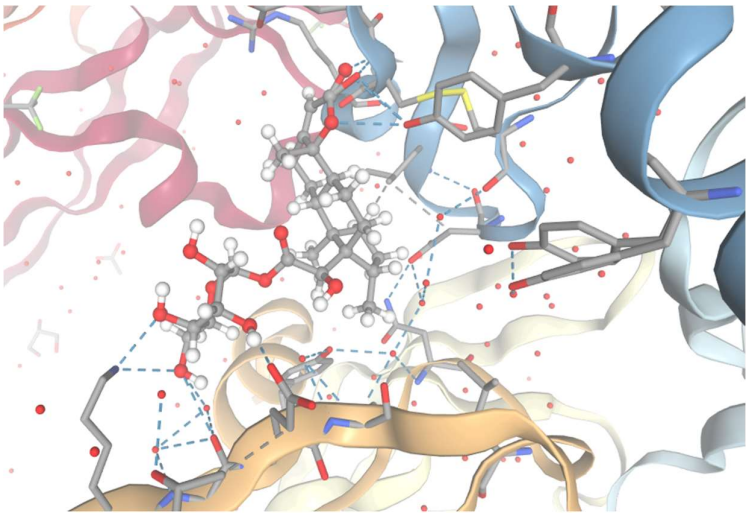

Figure 3. VEGF-A Protein Docking study of the Stevioside loaded hydrogel

3.5 Interaction with Transforming Growth Factor Beta 1 (TGF-β1)

3.5.1 Stevioside

Molecular docking analysis of stevioside with Transforming Growth Factor Beta 1 (TGF-β1; PDB ID: 1PY5) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.50 kcal/mol and an AC score of 244.38, indicating a thermodynamically favorable and stable interaction. The clustering of conformations around similar energy values suggested consistency of the predicted binding mode, while buried cavity prioritization indicated preferential occupation of energetically favorable regions within the protein structure. Interaction analysis revealed the presence of non-covalent interactions, including hydrogen bonding and hydrophobic contacts, which are consistent with the multiple hydroxyl groups and glycosidic linkages present in the stevioside molecule. Although specific residue-level interactions were not available, the observed interaction profile supports stable ligand accommodation within the binding cavity. TGF-β1 plays a pivotal role in wound healing by regulating fibroblast activation, collagen synthesis, and extracellular matrix remodeling. The favorable binding affinity of stevioside toward TGF-β1 suggests its potential to interact with pathways involved in tissue repair and skin regeneration. While molecular docking alone cannot confirm biological activity, these findings support the potential relevance of stevioside as a bioactive compound for wound-healing applications. Furthermore, incorporation of stevioside into a sustained-release hydrogel system may prolong its interaction with biological targets, thereby enhancing its therapeutic potential in skin tissue regeneration.

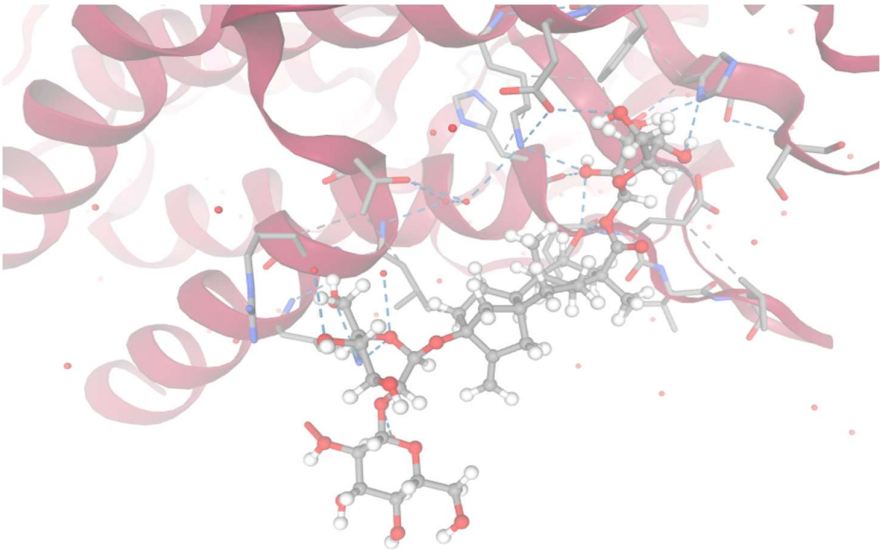

Figure 4. TGF-β1 Protein Docking study of the Stevioside molecule

3.5.2 Pluronic F127/ Agar Hydrogel

Molecular docking analysis of the Pluronic F127/Agar hydrogel-associated ligand system with Transforming Growth Factor Beta 1 (TGF-β1; PDB ID: 1PY5) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformations were identified within Cluster 0, with Member 1 exhibiting a binding free energy (ΔG) of −7.44 kcal/mol and an AC score of 82.85, while Member 2 showed a ΔG of −6.23 kcal/mol and an AC score of 105.89. These negative ΔG values indicate thermodynamically favorable interactions and suggest a consistent binding pattern within energetically favorable regions of the protein structure. Interaction analysis revealed the presence of non-covalent interactions, including hydrogen bonding and hydrophobic contacts, arising from the hydroxyl and ether functional groups of the Pluronic F127 and agar components. Although

specific residue-level interactions were not available, these interactions likely contribute to stabilization of the ligand system within the binding cavity. TGF-β1 is a key regulator of wound healing, controlling fibroblast proliferation, collagen synthesis, and extracellular matrix remodeling. The observed docking results suggest that the hydrogel components possess moderate affinity toward TGF-β1 under in silico conditions. However, the primary function of the Pluronic F127/Agar hydrogel is not direct protein modulation but rather to serve as a biocompatible drug delivery matrix providing thermoresponsive gelation, moisture retention, and sustained release of therapeutic agents. Therefore, while the docking results indicate favorable protein compatibility, the principal contribution of the hydrogel to skin regeneration is expected to arise from its supportive biomaterial properties and controlled drug delivery performance.

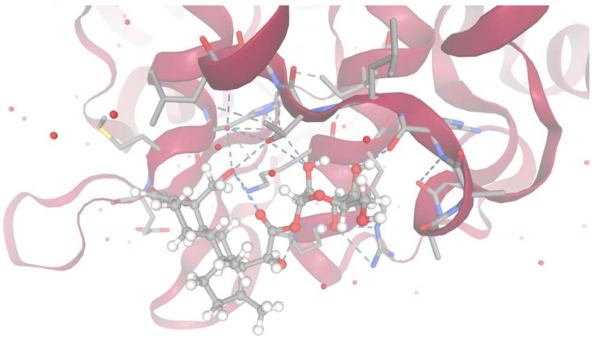

Figure 5. TGF-β1 Protein Docking study of the Pluronic F127/Agar hydrogel

3.5.3 Stevioside Loaded Hydrogel

Molecular docking analysis of the stevioside-loaded Pluronic F127/Agar hydrogel system with Transforming Growth Factor Beta 1 (TGF-β1; PDB ID: 1PY5) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.30 kcal/mol and an AC score of 103.28, indicating a thermodynamically favorable and stable interaction. The clustering of conformations around similar energy values suggested consistency of the predicted binding mode, while buried cavity prioritization indicated preferential occupation of energetically favorable regions within the protein structure. Interaction analysis revealed the presence of general non-covalent interactions, including hydrogen bonding and hydrophobic contacts arising from the hydroxyl and ether functional groups present in stevioside, Pluronic F127, and agar. Although specific residue-level interactions were not available, these interactions likely contribute to stabilization of the ligand system within the binding cavity. TGF-β1 is a critical regulator of wound healing, mediating fibroblast activation, collagen synthesis, and extracellular matrix remodeling. The observed binding affinity suggests that the composite hydrogel system possesses moderate interaction capability with TGF-β1 under in silico conditions. However, the primary therapeutic function of the hydrogel is not direct protein modulation but the controlled and sustained delivery of stevioside. By providing localized drug retention and prolonged release, the Pluronic F127/Agar matrix may enhance the duration of stevioside exposure to biological targets involved in tissue repair. Collectively, these findings support the potential application of the stevioside-loaded hydrogel as a wound-healing platform, where favorable protein interaction is complemented by sustained drug delivery and biomaterial-mediated support for skin regeneration.

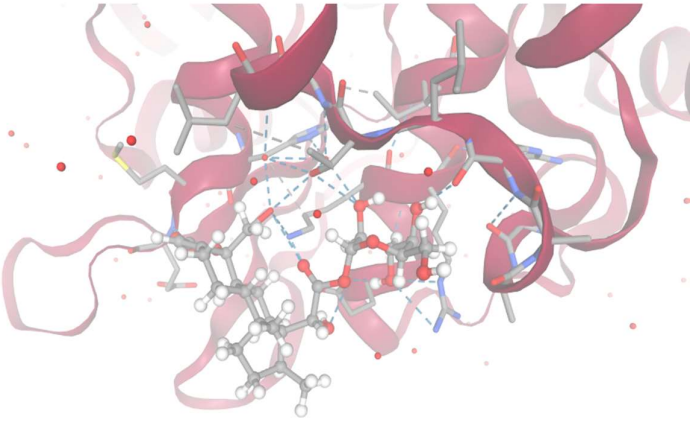

Figure 6. TGF-β1 Protein Docking study of the Stevioside loaded hydrogel

3.6 Interaction with Target Protein – Matrix Metalloproteinase-9 (MMP-9)

3.6.1 Stevioside

Molecular docking analysis of stevioside with Matrix Metalloproteinase-9 (MMP-9; PDB ID: 5TH6) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −8.55 kcal/mol and an AC score of 220.03, indicating a strong and thermodynamically favorable interaction with the target protein. The clustering of conformations around similar energy values suggested stability and reproducibility of the predicted binding mode. Interaction analysis revealed the presence of non-covalent interactions, including hydrogen bonding and hydrophobic contacts, which are consistent with the multiple hydroxyl and glycosidic functional groups present in stevioside. Although specific residue-level interactions were not available, these interactions likely contribute to stabilization of the ligand within the protein binding cavity. MMP-9 is a key regulator of extracellular matrix degradation and remodeling during wound healing, and its controlled activity is essential for maintaining the balance between tissue breakdown and regeneration. The strong binding affinity observed for stevioside suggests its potential to interact with MMP-9-mediated pathways involved in tissue repair. While molecular docking alone cannot confirm protein inhibition or activation, the results indicate a stable and energetically favorable interaction, supporting the potential relevance of stevioside in wound healing and skin regeneration applications through modulation of extracellular matrix remodeling processes.

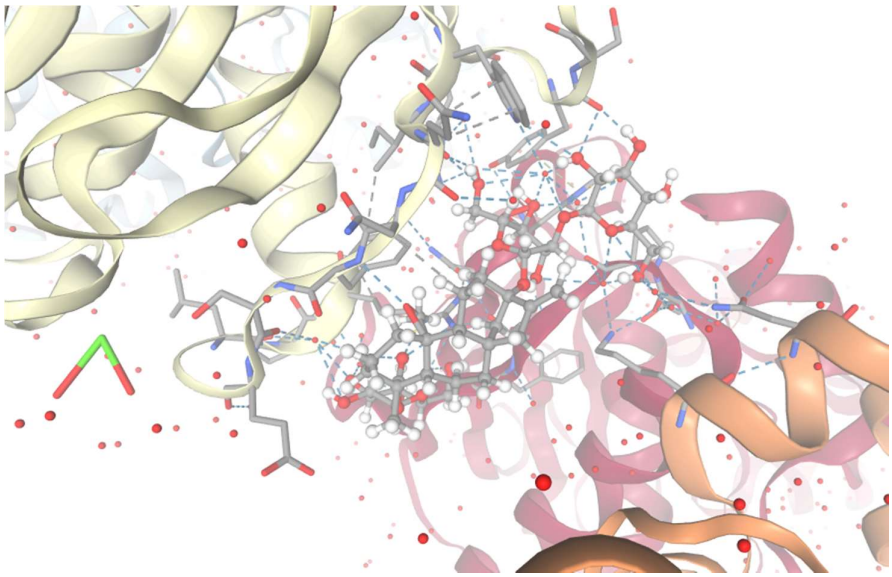

Figure 7. MMP-9 Protein Docking study of the Stevioside molecule

3.6.2 Pluronic F127/ Agar Hydrogel

Molecular docking analysis of the Pluronic F127/Agar hydrogel-associated ligand system with Matrix Metalloproteinase-9 (MMP-9; PDB ID: 5TH6) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.67 kcal/mol and an AC score of 77.86, indicating a thermodynamically favorable but moderate interaction with the target protein. The consistency of clustered conformations suggested stability of the predicted binding mode. Interaction analysis revealed the presence of non-covalent interactions, including hydrogen bonding and hydrophobic contacts, contributing to stabilization of the ligand–protein complex. The docking output indicated the involvement of the amino acid residue ILE137 within the binding region, confirming the ability of the hydrogel-associated ligand system to engage with the protein cavity. The interaction profile is consistent with the hydroxyl and ether functional groups present in the Pluronic F127 and agar components, which facilitate polar interactions and weak hydrophobic contacts. MMP-9 is a key enzyme involved in extracellular matrix degradation and remodeling during wound healing. Although the hydrogel components exhibited moderate affinity toward MMP-9, their primary role is not direct protein modulation but rather functioning as a biocompatible drug delivery matrix. The thermoresponsive nature, structural stability, and moisture-retention capacity of the Pluronic F127/Agar hydrogel support the wound-healing environment and facilitate sustained delivery of therapeutic agents. Therefore, the docking results suggest biological compatibility with MMP-9 while reinforcing that the principal contribution of the hydrogel to skin regeneration arises from its supportive biomaterial properties and controlled drug-release capabilities.

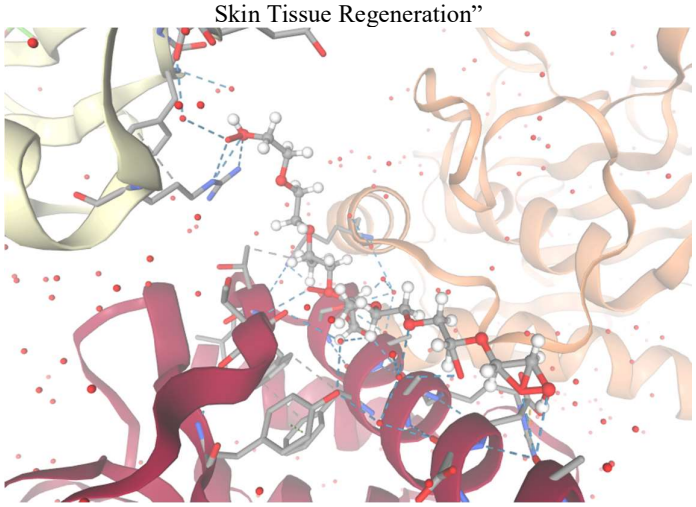

Figure 8. MMP-9 Protein Docking study of the Pluronic F127/Agar hydrogel

3.6.3 Stevioside Loaded Hydrogel

Molecular docking analysis of the stevioside-loaded Pluronic F127/Agar hydrogel system with Matrix Metalloproteinase-9 performed using th Attracting Cavities binding conformatio exhibiting binding fi −7.13 to −7.38 kcal/ for Member 3 (ΔG = The presence of mul energy values indica pattern, suggesting interaction with the revealed the involv ILE137 (chain C), within the MMP-9 b was characterized b including hydrogen arising from the hy present in stevioside, Pluronic F127, and agar. Although detailed residue-level mapping was not available, these interactions likely contribute to stabilization of the is a key enzyme degradation and ng, and balanced for effective tissue ffinity suggests that y interact favorably wever, the primary lies in providing evioside through the prolonging drug drogel may enhance involved in tissue upport the potential hydrogel in wound ombining favorable olled drug-release

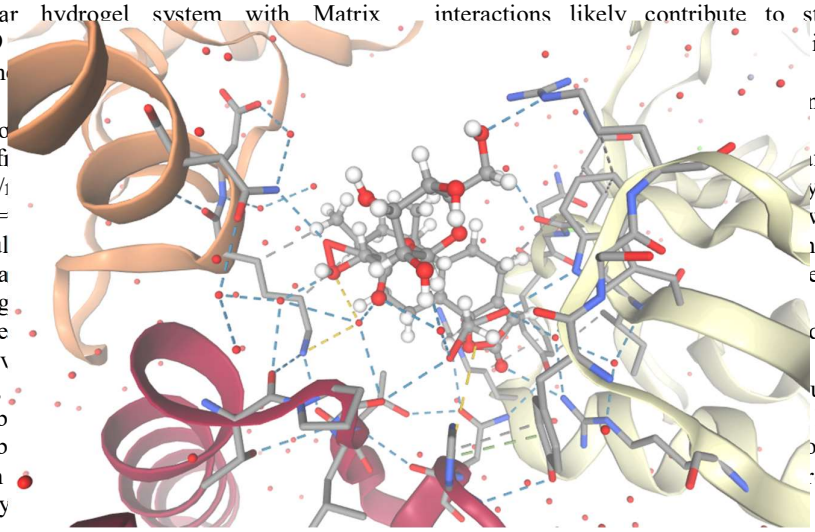

Figure 9. MMP-9 Protein Docking study of the Stevioside loaded hydrogel

3.7 Interaction with Target Protein – Interleukin-1 Beta (IL-1β)

3.7.1 Stevioside

Molecular docking analysis of stevioside with Interleukin-1 Beta (IL-1β; PDB ID: 6Y8M) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −6.25 kcal/mol and an AC score of 236.42, indicating a thermodynamically favorable and stable interaction with the target protein. The clustering of conformations around similar energy values suggested consistency and reproducibility of the predicted binding mode. Interaction analysis revealed the presence of non-covalent interactions, including hydrogen bonding and hydrophobic contacts, which are consistent with the multiple hydroxyl and glycosidic functional groups present in stevioside. Although specific amino acid residues and interaction distances were not available, these interactions likely contribute to stabilization of the ligand within the protein binding cavity. IL-1β is a key pro-inflammatory cytokine involved in regulating inflammatory responses during wound healing, and its controlled modulation is essential for preventing prolonged inflammation and promoting tissue repair. The observed binding affinity suggests that stevioside may interact with IL-1β-associated pathways relevant to skin regeneration. However, molecular docking provides only a predictive assessment of binding behavior and does not confirm protein inhibition or activation. Therefore, the biological significance of this interaction requires further experimental validation. Nevertheless, the stable and energetically favorable interaction observed supports the potential relevance of stevioside in wound healing and skin regeneration applications, particularly through its possible involvement in inflammatory regulation.

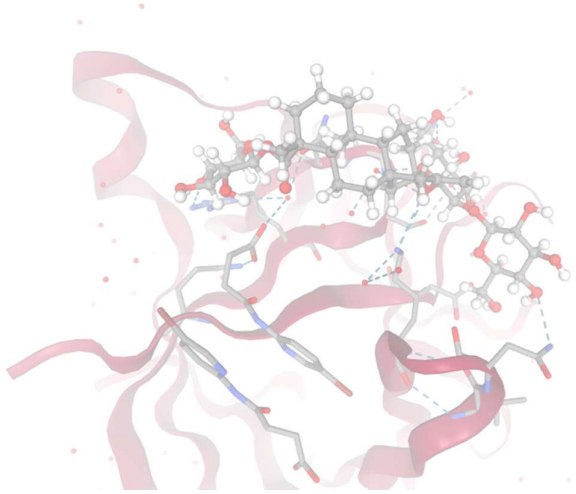

Figure 10. IL-1β Protein Docking study of the Stevioside molecule

3.7.2 Pluronic F127/ Agar Hydrogel

Molecular docking analysis of the Pluronic F127/Agar hydrogel-associated ligand system with Interleukin-1 Beta (IL-1β; PDB ID: 6Y8M) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of +5.84 kcal/mol and an AC score of 273.68. In contrast to negative ΔG values, the positive binding free energy indicates that the interaction is thermodynamically unfavorable under the simulated conditions, suggesting minimal or negligible binding affinity between the hydrogel-associated ligand representation and IL-1β. Although clustering of conformations was observed, the positive energy value indicates the absence of a stable ligand–protein complex. Interaction visualization suggested the presence of weak non-covalent contacts, including hydrogen bonding and hydrophobic interactions; however, no specific amino acid residues were identified, and the available data did not support the existence of a well-defined binding mode. The Pluronic F127 and agar components contain hydroxyl and ether functional groups capable of forming polar interactions, but these interactions appear insufficient to stabilize the complex. IL-1β is a key pro-inflammatory cytokine involved in wound healing and inflammatory regulation; however, the docking results indicate that the hydrogel matrix itself is unlikely to directly interact with or modulate this target. This finding is consistent with the intended role of the Pluronic F127/Agar hydrogel as a biocompatible drug delivery platform rather than a bioactive ligand. Therefore, its contribution to skin regeneration is primarily attributed to its moisture-retention capacity, structural support, and ability to provide controlled and localized delivery of therapeutic agents, rather than direct molecular interaction with IL-1β.

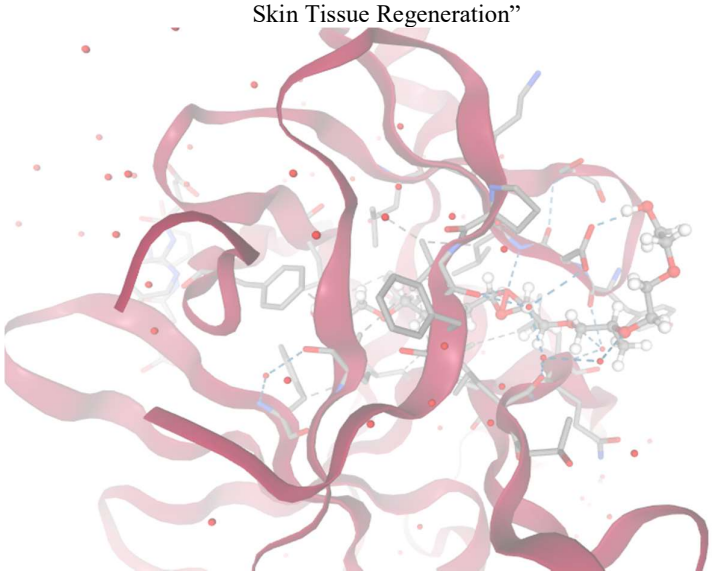

Figure 11. IL-1β Protein Docking study of the Pluronic F127/Agar hydrogel

3.7.3 Stevioside Loaded Hydrogel

Molecular docking analysis of the stevioside-loaded Pluronic F127/Agar hydrogel system with Interleukin-1 Beta (IL-1β; PDB ID: 6Y8M) was performed using the SwissDock server employing the Attracting Cavities 2.0 algorithm. The most favorable binding conformation was identified in Cluster 0 (Member 1), exhibiting a binding free energy (ΔG) of −4.31 kcal/mol and an AC score of 154.13, indicating a weak to moderate thermodynamically favorable interaction with the target protein. The clustering of conformations suggested some degree of reproducibility in the predicted binding mode; however, the relatively low magnitude of the binding energy indicates limited stability of the ligand–protein complex. Interaction analysis revealed the presence of general non-covalent interactions, including hydrogen bonding and hydrophobic contacts, arising from the hydroxyl and ether functional groups present in stevioside, Pluronic F127, and agar. However, no specific amino acid residues or detailed interaction parameters were identified, indicating the absence of a well-defined binding orientation within the protein cavity. IL-1β is a key pro-inflammatory cytokine involved in regulating inflammatory responses during wound healing, and its modulation is important for effective tissue repair. The weak binding affinity observed suggests that the composite hydrogel system is unlikely to directly influence IL-1β activity through strong molecular interactions. Instead, its therapeutic benefit is expected to arise primarily from the controlled and sustained release of stevioside, facilitated by the Pluronic F127/Agar matrix. Therefore, the hydrogel functions predominantly as a biocompatible drug delivery platform that enhances localized drug retention and prolonged therapeutic exposure, rather than as a direct modulator of IL-1β-mediated inflammatory pathways.

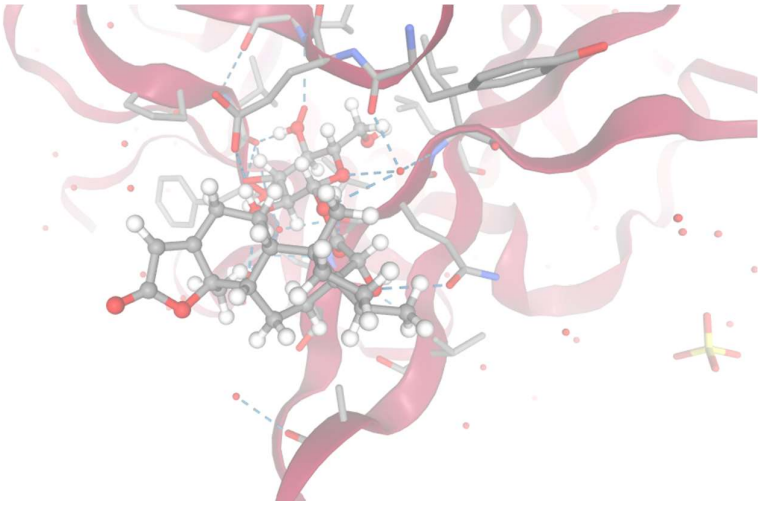

Figure 12. IL-1β Protein Docking study of the Stevioside loaded hydrogel.

Table 2. Molecular Docking Summary of Stevioside, polymeric Hydrogel, and Stevioside Loaded hydrogel

System	Target Protein	PDB ID	Cluster	Best Member	ΔG (kcal/mol)	AC Score	Binding Interpretation
Stevioside	VEGF-A	3QTK	0	1	−8.13	231.39	Strong binding
Stevioside	MMP-9	5TH6	0	1	−8.55	220.03	Strong binding
Stevioside	IL-1β	6Y8M	0	1	−6.25	236.42	Moderate binding
Pluronic F127/Agar	MMP-9	5TH6	0	1	−6.67	77.86	Moderate binding
Pluronic F127/Agar	IL-1β	6Y8M	0	1	+5.84	273.68	Unfavorable binding
Stevioside-loaded Hydrogel	MMP-9	5TH6	0	3	−7.38	96.69	Moderate–strong binding
Stevioside-loaded Hydrogel	IL-1β	6Y8M	0	1	−4.31	154.13	Weak binding

Table 3. Binding Interaction Analysis (Residue-Level Information)

System	Target Protein	PDB ID	Identified Residues	Interaction Type	Remarks
Stevioside	VEGF-A	3QTK	ASP12 (observed)	Hydrogen bonding / polar	Limited residue data available
Stevioside	MMP-9	5TH6	Not explicitly reported	General non-covalent	Residues not defined in dataset
Stevioside	IL-1β	6Y8M	Not explicitly reported	General non-covalent	Residues not defined
Pluronic F127/Agar	MMP-9	5TH6	Not explicitly reported	Weak polar + hydrophobic	Polymer interaction
Pluronic F127/Agar	IL-1β	6Y8M	Not reported	Weak/transient	Unstable interaction
Stevioside-loaded Hydrogel	MMP-9	5TH6	ILE137	Hydrophobic / backbone interaction	Confirmed residue
Stevioside-loaded Hydrogel	IL-1β	6Y8M	Not reported	Weak non-specific	No residue mapping

Table 4. Comparative Functional Interpretation in Skin Regeneration

System	Biological Role	Target Protein	Functional Relevance	Overall Contribution
Stevioside	Bioactive compound	VEGF-A	Promotes angiogenesis	Strong therapeutic potential
Stevioside	Bioactive compound	MMP-9	ECM remodeling regulation	Supports wound healing
Stevioside	Bioactive compound	IL-1β	Inflammation modulation	Moderate anti-inflammatory role
Pluronic F127/Agar	Drug carrier	MMP-9	Minimal direct interaction	Structural support
Pluronic F127/Agar	Drug carrier	IL-1β	No binding	Passive role
Stevioside-loaded Hydrogel	Combined system	MMP-9	Sustained interaction	Enhanced efficacy
Stevioside-loaded Hydrogel	Combined system	IL-1β	Weak interaction	Controlled release effect

Table 5. Binding Strength Classification

ΔG Range (kcal/mol)	Binding Strength	Interpretation
≤ −8.0	Strong	High affinity, stable complex
−6.0 to −8.0	Moderate	Favorable interaction
−4.0 to −6.0	Weak	Limited stability
> 0	Unfavorable	No stable binding

4.CONCLUSION

The study demonstrated that agar concentration effectively modulates the thermoresponsive behavior, structural integrity, and drug delivery performance of Pluronic F127 hydrogels. The optimized formulation exhibited favorable gelation properties and strong network formation. Molecular docking revealed that stevioside possesses notable affinity toward skin regeneration-related proteins, particularly VEGF-A and MMP-9, while the hydrogel matrix primarily functions as a controlled drug delivery platform. Overall, the

optimized hydrogel enables sustained stevioside release and represents a promising, clinically relevant system for skin tissue regeneration and wound healing applications.

5.CONFLICT OF INTEREST: None

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