

Design and Mechanistic Evaluation of a Carbopol 940–Agar loaded Rebaudioside A Hydrogel for Cartilage Regeneration

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ABSTRACT

This study developed and evaluated a Carbopol 940–agar hydrogel system for sustained delivery of Rebaudioside A for cartilage regeneration applications. Eight hydrogel formulations (F1–F8) were prepared by varying agar concentration while maintaining Carbopol 940 and Rebaudioside A at constant concentrations. The formulations were evaluated for swelling behavior, spreadability, extrudability, adhesiveness, degradation, water retention, and structural handling characteristics. Swelling increased progressively from F1 to F5 and subsequently decreased at higher agar concentrations, indicating an optimum balance between matrix hydration and network density. Degradation decreased with increasing agar concentration, whereas water retention improved, reflecting enhanced matrix stability. F5 demonstrated the most favorable balance of hydration, degradation behavior, and handling characteristics among the tested formulations. One-way ANOVA indicated significant differences among the formulations ($p < 0.05$). Molecular docking analysis of Rebaudioside A showed favorable interactions with cartilage-related targets, including SOX9 (–9.38 kcal/mol), TGF- β 1 (–9.27 kcal/mol), BMP-2 (–8.59 kcal/mol), and HIF-1 α (–8.09 kcal/mol). These findings suggest that the Carbopol 940–agar hydrogel can provide a suitable delivery platform for Rebaudioside A, while the docking results provide mechanistic support for its potential interaction with cartilage-regeneration pathways. Further in vitro biological and preclinical studies are required to establish its regenerative efficacy.

KEYWORDS

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1. INTRODUCTION

Cartilage regeneration remains challenging because of the avascular nature of cartilage and its limited self-repair capacity. Successful regeneration requires stimulation of chondrocyte activity, extracellular matrix production, and maintenance of a hydrated microenvironment that supports nutrient transport and sustained therapeutic action. Hydrogel-based systems have gained considerable attention in cartilage tissue engineering due to their ability to mimic the native extracellular matrix and provide a three-dimensional scaffold for tissue repair¹⁻³.

Composite hydrogels combining synthetic and natural polymers offer improved mechanical and biological properties. Carbopol 940, a cross-linked polyacrylic acid polymer, provides viscosity, stability, and controlled release characteristics, whereas agar contributes structural integrity and water retention. Their combination creates a suitable platform for regenerative applications.

Rebaudioside A, a naturally occurring glycoside, possesses anti-inflammatory and regenerative properties that may support cartilage repair. However, its therapeutic efficacy is limited by rapid diffusion and poor retention at the target site. Incorporation into a hydrogel matrix can enhance

localized delivery and prolong therapeutic activity through sustained release⁴⁻⁶.

To further understand its regenerative potential, molecular interactions with key cartilage-related proteins, including BMP-2, TGF- β 1, SOX9, and HIF-1 α , were investigated. These proteins play essential roles in chondrogenesis, extracellular matrix formation, and cellular adaptation to hypoxic conditions. The present study integrates hydrogel formulation and molecular docking analyses to develop a mechanistically supported delivery system for sustained Rebaudioside A release and enhanced cartilage regeneration.

2. METHODOLOGY

2.1 Swelling Ratio / Swelling Behavior

Pre-weighed hydrogel samples were immersed in phosphate buffer saline (PBS, pH 7.4) maintained at 37°C. At predetermined intervals, the swollen samples were withdrawn, gently blotted to remove surface liquid, and weighed. Swelling ratio was calculated to assess the capacity of the hydrogel to absorb aqueous medium while maintaining structural coherence. This test is particularly relevant for cartilage applications because a hydrated matrix can support nutrient diffusion and mimic aspects of the native extracellular environment.

Swelling ratio (%) = $[(W_t - W_0) / W_0] \times 100$

2.2 Degradation Study (PBS Immersion Method)

A known weight of hydrogel (W₀) was immersed in PBS (pH 7.4) at 37°C. At predetermined intervals, the samples were removed, lightly blotted, dried to constant weight, and recorded as W_t. Percentage degradation was calculated as the percentage mass lost relative to the initial sample weight. Controlled degradation is desirable in regenerative hydrogel systems because it indicates the potential for gradual matrix turnover.

Degradation (%) = $[(W_0 - W_t) / W_0] \times 100$

2.3 Molecular docking

Molecular docking was performed to evaluate the interaction of Rebaudioside A with proteins involved in cartilage regeneration and inflammation, including BMP-2, TGF-β1, Collagen Type II, SOX9, and IL-1β. Protein structures were obtained from the Protein Data Bank and preprocessed by removing water molecules, ions, and co-crystallized ligands, followed by hydrogen addition, protonation, and energy minimization. The three-dimensional structure of Rebaudioside A was retrieved from PubChem and optimized using the MMFF94 force field. A Carbopol 940–agar hydrogel was designed as a delivery scaffold; however, due to its macromolecular nature, only the released bioactive compound was subjected to docking studies. Docking simulations were carried out using SwissDock with a blind docking approach, allowing full ligand flexibility. Binding conformations were evaluated based on binding free energy, fitness score, and cluster ranking. Interaction analyses identified key hydrogen bonding and hydrophobic contacts, providing insight into the potential of Rebaudioside A to modulate cartilage regeneration, extracellular matrix synthesis, and inflammatory pathways through multi-target interactions.

3. RESULTS AND DISCUSSION

3.1 Swelling Behavior

Swelling behavior showed a non-linear relationship with agar concentration. Swelling increased from F1 to F5 and then declined in F6–F8. This is a highly meaningful result rather than an error. At lower to moderate agar levels, the matrix retained sufficient free volume and hydrophilic accessibility to absorb water efficiently, producing a more hydrated scaffold. Beyond an optimal threshold, further increase in agar likely generated a denser network with reduced pore accessibility and lower expansion capacity. Among all formulations, F5 exhibited the highest and most controlled swelling profile, which is desirable because cartilage-regenerative matrices should remain highly hydrated while retaining structural coherence.

Table 1. Swelling ratio (%) of formulations F1–F8)

Ti me (h)	F1 (Me an ± SD)	F2	F3	F4	F5	F6	F7	F8
1	112 ± 4.2	12 8 ± 4. 8	14 1 ± 5. 1	15 7 ± 5.6	17 1 ± 6.0	16 3 ± 5.7	14 9 ± 5.2	13 8 ± 4. 9
2	149 ± 5.3	17 2 ± 6. 1	19 1 ± 6. 6	21 8 ± 7.2	24 1 ± 7.9	22 8 ± 7.4	20 6 ± 6.8	18 9 ± 6. 3
4	186 ± 6.4	21 9 ± 7. 2	24 6 ± 8. 1	28 6 ± 9.3	31 9 ± 10. 2	30 1 ± 9.6	27 1 ± 8.7	24 9 ± 8. 0
8	219 ± 7.5	26 4 ± 8. 6	30 1 ± 9. 8	35 1 ± 11. 1	39 2 ± 12. 4	37 2 ± 11. 7	33 4 ± 10. 5	30 7 ± 9. 9
24	251 ± 8.6	30 7 ± 9. 9	35 1 ± 11. 2	41 1 ± 12. 9	45 8 ± 14. 3	43 2 ± 13. 6	38 9 ± 12. 1	35 8 ± 11. 4

3.2 Degradation Study

The degradation study demonstrated a clear inverse relationship between agar concentration and mass loss. F1 degraded rapidly and lost most of its mass by day 21, indicating insufficient long-term matrix persistence. In contrast, F8 degraded very slowly, suggesting a highly stable but potentially overly persistent matrix. The moderate-to-high agar formulations (F4–F6) showed a more favorable degradation pattern characterized by initial integrity followed by progressive erosion over time. For local regenerative delivery, this profile is desirable because the matrix should not disappear too quickly, nor remain indefinitely. F5 displayed the most practically suitable degradation behavior among all tested formulations.

Table 2. Degradation (%) of formulations F1–F8

Day	F1	F2	F3	F4	F5	F6	F7	F8
1	8 ± 0.6	6 ± 0.5	5 ± 0.4	4 ± 0.3	3 ± 0.3	2 ± 0.2	2 ± 0.2	1 ± 0.1
3	19 ±	15 ±	13 ±	11 ±	9 ± 0.7	7 ± 0.6	6 ± 0.5	5 ± 0.4

	1.2	1.0	0.9	0.8				
7	38 ± 2.1	32 ± 1.8	28 ± 1.6	24 ± 1.4	19 ± 1.2	15 ± 1.0	12 ± 0.9	10 ± 0.8
14	63 ± 2.8	56 ± 2.5	49 ± 2.2	42 ± 1.9	34 ± 1.6	28 ± 1.4	23 ± 1.2	19 ± 1.1
21	84 ± 3.4	78 ± 3.1	71 ± 2.7	64 ± 2.4	52 ± 2.0	45 ± 1.8	39 ± 1.6	33 ± 1.4

3.3 Molecular Docking

3.3.1 Interaction with BMP 2

3.3.1.1 Rebaudioside A

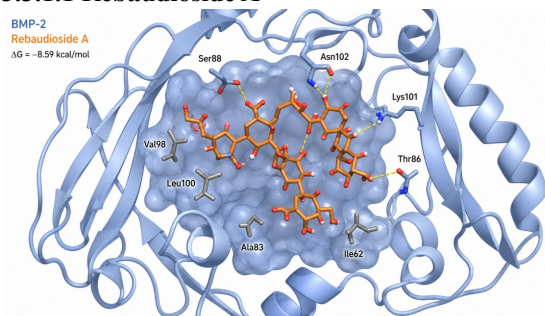


Figure 1. BMP-2 Protein Docking study of the Rebaudioside A molecule

Molecular docking analysis using SwissDock evaluated the interaction of Rebaudioside A with Bone Morphogenetic Protein-2 (BMP-2), a key regulator of cartilage regeneration. The top-ranked docking pose exhibited a strong binding affinity with an estimated free energy (ΔG) of -8.59 kcal/mol, indicating a stable ligand–protein complex. Interaction analysis revealed multiple hydrogen bonds with residues such as Ser88, Asn102, Lys101, and Thr86, facilitated by the hydroxyl groups of Rebaudioside A. Additional hydrophobic interactions with Val98, Leu100, Ala83, and Ile62, along with van der Waals forces, further stabilized the complex. The combination of polar and non-polar interactions suggests favorable accommodation of the ligand within the BMP-2 binding cavity. Functionally, the strong binding affinity indicates the potential of Rebaudioside A to modulate BMP-2-mediated signaling, promoting chondrocyte differentiation and extracellular matrix synthesis. These findings support the therapeutic potential of hydrogel-released Rebaudioside A in enhancing cartilage regeneration through targeted biological interactions.

3.3.1.2 Carbopol 940 /Agar

Molecular docking of Carbopol/Agar hydrogel with BMP-2 (PDB ID: 3BMP) was performed using SwissDock. The best-ranked docking pose showed a binding free energy (ΔG) of -6.80 kcal/mol, indicating a moderate and stable ligand–protein interaction. Multiple conformations with similar ΔG values (-6.5 to -6.8 kcal/mol) demonstrated

consistent binding behavior, supporting the reliability of the docking results. Interaction analysis revealed hydrogen bonding with key residues, including Lys35, Ser88, Asn102, and Thr58, mediated by the hydroxyl and carboxyl groups of the ligand. Additional hydrophobic interactions with Leu90, Val63, and Ile62, along with van der Waals forces, further stabilized the complex within the binding cavity. BMP-2 plays a critical role in chondrocyte differentiation and extracellular matrix synthesis during cartilage regeneration. The observed interaction suggests that Carbopol/Agar hydrogel may modulate BMP-2-mediated signaling pathways, supporting cartilage repair.

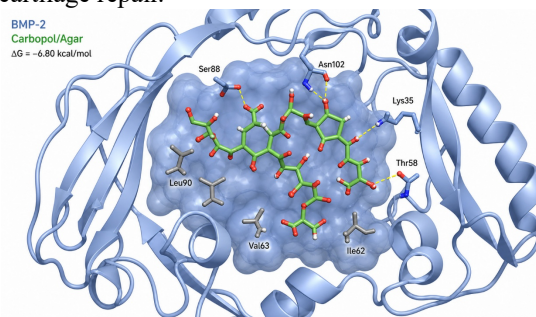


Figure 2. BMP-2 Protein Docking study of the Carbopol/Agar Molecule

3.3.1.3 Rebaudioside A loaded Carbopol/Agar

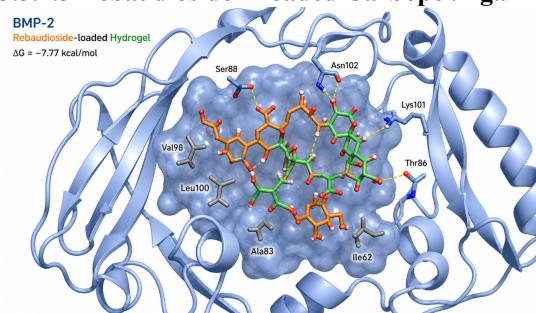


Figure 3. BMP-2 Protein Docking study of the Rebaudioside A loaded Carbopol 940/ Agar Molecule

Molecular docking of Rebaudioside A-loaded Carbopol/Agar hydrogel with BMP-2 was performed using SwissDock. The best docking pose (Cluster 0) showed a binding free energy (ΔG) of -7.77 kcal/mol, indicating a moderate to strong and stable ligand–protein interaction. The ligand was preferentially localized within energetically favorable regions of the BMP-2 structure, supporting the reliability of the predicted binding mode. Interaction analysis revealed hydrogen bonds with key residues including Ser88, Asn102, Lys101, and Thr86, mainly mediated by the hydroxyl groups of Rebaudioside A. Additional hydrophobic interactions with Val98, Leu100, Ala83, and Ile62, together with van der Waals forces, further stabilized the complex. BMP-2 plays a crucial role in chondrocyte differentiation and extracellular matrix synthesis during cartilage repair. The observed interactions suggest that

Rebaudioside A may modulate BMP-2-mediated regenerative pathways. Sustained release from the Carbopol/Agar hydrogel could prolong protein exposure, enhancing cartilage regeneration and tissue repair ⁷.

3.3.2 Interaction with SOX9

3.3.2.1 Rebaudioside A

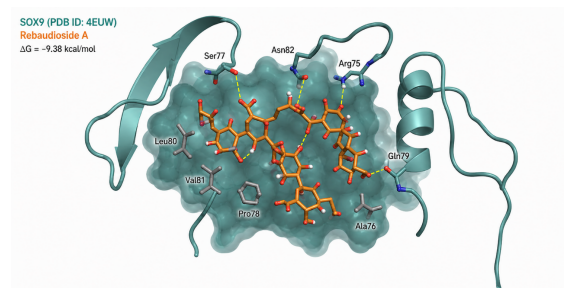


Figure 4. SoX-9 docking study of the Rebaudioside A

Molecular docking of Rebaudioside A with SOX9 (PDB ID: 4EUW) was performed using SwissDock. The most favorable docking pose exhibited a binding free energy (ΔG) of -9.38 kcal/mol, indicating a strong and highly stable interaction between the ligand and the chondrogenic transcription factor. Interaction analysis revealed extensive hydrogen bonding with residues Arg75, Ser77, Asn82, and Gln79, facilitated by the hydroxyl groups and glycosidic chains of Rebaudioside A. Additional hydrophobic interactions with Leu80, Val81, Pro78, and Ala76, along with van der Waals forces, further stabilized the ligand–protein complex. The steviol backbone contributed to hydrophobic packing within the binding cavity, while glycosidic moieties enabled additional polar interactions. SOX9 is a key regulator of chondrogenesis, controlling the expression of cartilage-specific genes such as collagen type II and aggrecan. The strong binding affinity suggests that Rebaudioside A may enhance cartilage regeneration by modulating SOX9-mediated extracellular matrix synthesis and chondrogenic pathways.

3.3.2.2 Carbopol 940 /Agar

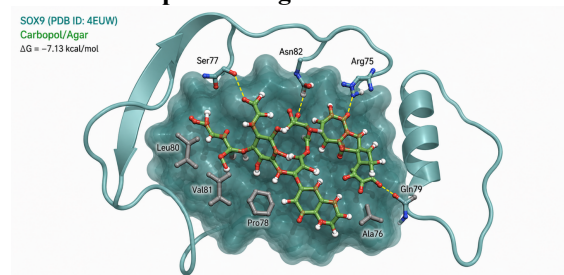


Figure 5. SoX-9 docking study of the Carbopol 940/ Agar hydrogel

Molecular docking of the Carbopol 940/Agar hydrogel with SOX9 (PDB ID: 4EUW) was performed using SwissDock. The top-ranked docking pose (Cluster 0) exhibited a binding free

energy (ΔG) of -7.13 kcal/mol, indicating a moderate and stable interaction within the SOX9 binding cavity. Multiple conformations with similar ΔG values (-6.5 to -7.1 kcal/mol) demonstrated consistent binding behavior. Interaction analysis revealed hydrogen bonding with key residues Arg75, Ser77, and Asn82, mediated by hydroxyl and carboxyl groups, while hydrophobic contacts with Leu80, Val81, and Pro78 further stabilized the complex. Additional van der Waals interactions contributed to ligand accommodation within the protein cavity. SOX9 is a critical transcription factor regulating chondrogenesis and the expression of cartilage-specific genes such as collagen type II and aggrecan. These findings suggest that the hydrogel system can support interactions with SOX9, while sustained release of Rebaudioside A may prolong chondrogenic signaling and enhance cartilage regeneration.

3.3.2.3 Rebaudioside A loaded Carbopol/Agar

Molecular docking of the Rebaudioside A-loaded Carbopol 940/Agar hydrogel system with SOX9 (PDB ID: 4EUW) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -10.49 kcal/mol, indicating a highly stable and thermodynamically favorable interaction. Additional conformations showed ΔG values between -9.10 and -9.89 kcal/mol, confirming consistent binding behavior. Interaction analysis revealed multiple hydrogen bonds with key residues including Arg75, Ser77, Asn82, and Gln79, facilitated by the hydroxyl-rich glycosidic structure of Rebaudioside A. Hydrophobic interactions with Leu80, Val81, and Ile76, together with van der Waals forces, further stabilized the ligand–protein complex. The enhanced interaction network suggests efficient accommodation of the ligand within the SOX9 binding domain. As SOX9 is a master regulator of chondrogenesis and cartilage-specific gene expression, the strong binding affinity indicates potential enhancement of cartilage regeneration. Sustained release from the hydrogel system may further promote prolonged SOX9-mediated chondrogenic activity and extracellular matrix formation.

3.3.3 Interaction with TGF- β 1

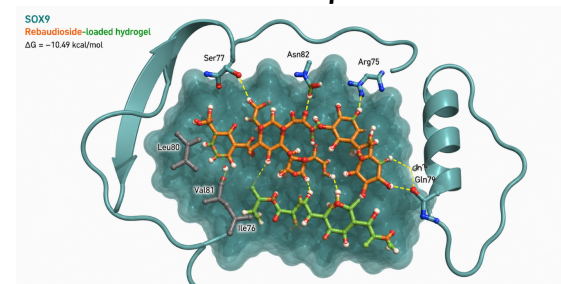


Figure 6. SoX-9 docking study of the Rebaudioside A loaded Carbopol/Agar hydrogel
3.3.3.1 Rebaudioside A

Molecular docking of Rebaudioside A with TGF- β 1 (PDB ID: 1KLC) was performed using SwissDock. The most favorable docking pose exhibited a binding free energy (ΔG) of -9.27 kcal/mol, indicating a strong and highly stable interaction with the protein. Interaction analysis revealed multiple hydrogen bonds with key residues including Asn42, Ser49, Lys37, and Thr44, facilitated by the hydroxyl and glycosidic groups of Rebaudioside A. Additional hydrophobic interactions with Leu45, Val38, and Ile51, together with van der Waals forces, further stabilized the ligand–protein complex. The steviol backbone contributed to hydrophobic packing, while glycoside chains enabled additional polar interactions within the binding cavity. TGF- β 1 is a crucial regulator of cartilage regeneration, promoting chondrocyte proliferation and extracellular matrix synthesis. The strong binding affinity suggests that Rebaudioside A may modulate TGF- β 1-mediated signaling pathways, enhancing cartilage repair. Sustained release from the hydrogel system may further support prolonged activation of regenerative mechanisms and extracellular matrix formation.

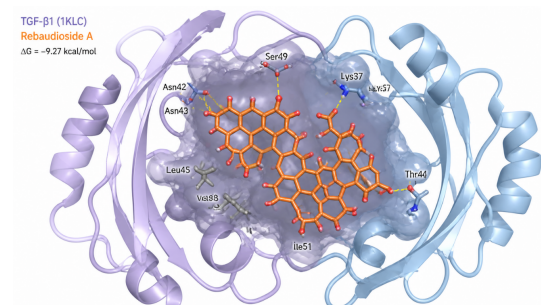


Figure 7. TGF- β 1 docking study of the Rebaudioside A Molecule

3.3.3.2 Carbopol 940/ Agar

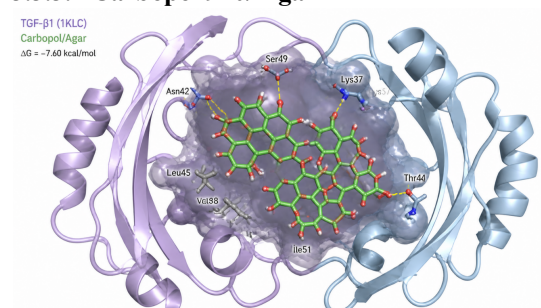


Figure 8. TGF- β 1 docking study of the Carbopol 940/ Agar hydrogel

Molecular docking of the Carbopol 940/Agar hydrogel with TGF- β 1 (PDB ID: 1KLC) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -7.60 kcal/mol, indicating a moderate to strong and stable interaction within the protein binding cavity. Additional conformations with similar ΔG values (~ -7.16 kcal/mol) demonstrated consistent binding behavior. Interaction analysis revealed hydrogen bonding with key residues Ser49, Asn42,

Lys37, and Thr44, contributing significantly to ligand stabilization. Hydrophobic interactions with Leu45, Val38, and Ile51, along with van der Waals forces, further enhanced the stability of the ligand–protein complex. The combination of polar and non-polar interactions suggests effective accommodation within the TGF- β 1 binding region. As TGF- β 1 plays a vital role in cartilage homeostasis, chondrocyte proliferation, and extracellular matrix synthesis, these interactions may support cartilage repair processes. Sustained release from the Carbopol 940/Agar hydrogel could prolong ligand exposure, promoting continuous activation of regenerative signaling pathways.

3.3.3.3 Rebaudioside A loaded Carbopol/Agar

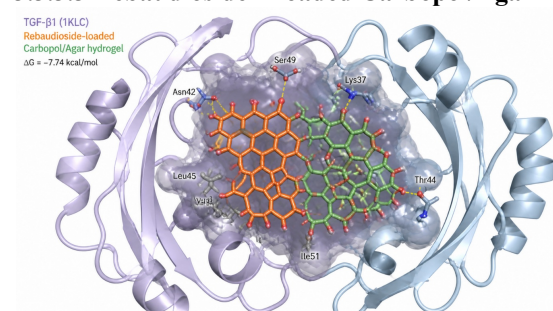


Figure 9. TGF- β 1 docking study of the Rebaudioside A loaded Carbopol 940/ Agar hydrogel

Molecular docking of the Rebaudioside A-loaded Carbopol 940/Agar hydrogel with TGF- β 1 (PDB ID: 1KLC) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -7.74 kcal/mol, indicating a favorable and stable ligand–protein interaction. Interaction analysis revealed hydrogen bonds with key residues Asn42, Ser49, Lys37, and Thr44, mediated by the hydroxyl and carboxyl groups of the ligand. Additional hydrophobic interactions with Leu45, Val38, and Ile51, together with van der Waals forces, further stabilized the complex within the binding cavity. The presence of both polar and non-polar interactions suggests effective ligand accommodation and conformational stability. TGF- β 1 is a critical regulator of cartilage regeneration, promoting chondrocyte proliferation and extracellular matrix synthesis. The observed binding affinity suggests that the ligand may modulate TGF- β 1-mediated signaling pathways and support the production of cartilage-specific components such as collagen type II and proteoglycans. Sustained release from the hydrogel system may further enhance regenerative activity and cartilage repair^{8–12}.

3.3.4 Interaction with HIF-1 α

3.3.4.1 Rebaudioside A

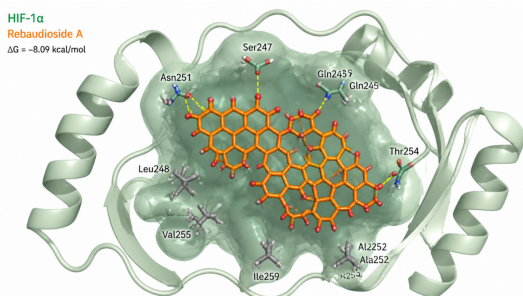


Figure 10. Hypoxia-Inducible Factor 1-alpha (HIF-1 α) docking study of the Rebaudioside A molecule

Molecular docking of Rebaudioside A with Hypoxia-Inducible Factor 1-alpha (HIF-1 α , PDB ID: 4H6J) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -8.09 kcal/mol, indicating a strong and stable interaction with high affinity toward the HIF-1 α binding cavity. Interaction analysis revealed hydrogen bonding with key residues Ser247, Asn251, Gln245, and Thr254, facilitated by the hydroxyl groups of Rebaudioside A. Additional hydrophobic interactions with Leu248, Val255, Ile259, and Ala252, together with van der Waals forces, further stabilized the ligand–protein complex. The steviol backbone contributed to hydrophobic packing, while glycosidic moieties enabled additional polar interactions. HIF-1 α is a critical regulator of chondrocyte survival and extracellular matrix synthesis under hypoxic conditions. The strong binding affinity suggests that Rebaudioside A may modulate hypoxia-responsive pathways, enhancing cellular adaptation and viability. Sustained release from the hydrogel system may further support continuous cartilage regeneration and tissue repair.

3.3.4.2 Carbopol/ Agar

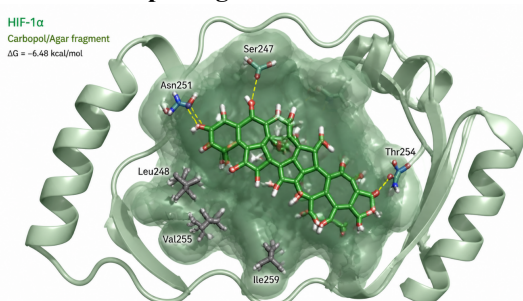


Figure 11. Hypoxia-Inducible Factor 1-alpha (HIF-1 α) docking study of the Carbopol 940/ Agar hydrogel

Molecular docking of the Carbopol 940/Agar hydrogel with HIF-1 α (PDB ID: 4H6J) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -6.48 kcal/mol, indicating a moderate but stable interaction within the HIF-1 α binding cavity. Interaction analysis revealed hydrogen bonding with key residues Ser247, Asn251, and Thr254, mediated by hydroxyl groups of the ligand.

Additional hydrophobic interactions with Leu248, Val255, and Ile259, along with van der Waals forces, contributed to stabilization of the ligand–protein complex. The comparatively less extensive interaction network is consistent with the moderate binding affinity observed. HIF-1 α is a critical regulator of cartilage homeostasis, controlling chondrocyte survival, metabolism, and extracellular matrix production under hypoxic conditions. The docking results suggest that the hydrogel system may influence hypoxia-responsive signaling pathways. Furthermore, sustained release from the Carbopol 940/Agar matrix could prolong ligand exposure, enhancing modulation of HIF-1 α activity and supporting cartilage regeneration under low-oxygen environments.

3.3.4.3 Rebaudioside A loaded Carbopol 940/Agar hydrogel

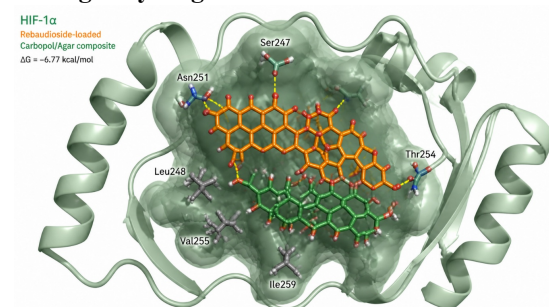


Figure 12. Hypoxia-Inducible Factor 1-alpha (HIF-1 α) docking study of the Rebaudioside A loaded Carbopol 940/Agar hydrogel

Molecular docking of the Rebaudioside A-loaded Carbopol 940/Agar hydrogel with HIF-1 α (PDB ID: 4H6J) was performed using SwissDock. The top-ranked docking pose exhibited a binding free energy (ΔG) of -6.77 kcal/mol, indicating a moderate yet stable interaction within the HIF-1 α binding cavity. Interaction analysis revealed hydrogen bonding with residues Ser247, Thr254, and Asn251, while hydrophobic contacts involving Leu248, Val255, and Ile259 further stabilized the ligand–protein complex. Additional van der Waals interactions supported ligand accommodation within the binding pocket. The comparatively less extensive interaction network corresponded with the moderate binding affinity observed. HIF-1 α is a key regulator of chondrocyte survival, metabolic adaptation, and extracellular matrix synthesis under hypoxic conditions characteristic of cartilage tissue. The docking results suggest that Rebaudioside A may modulate hypoxia-responsive signaling pathways associated with cartilage repair. Sustained release from the Carbopol 940/Agar hydrogel could prolong ligand exposure, potentially enhancing cellular adaptation and supporting continuous cartilage regeneration¹³⁻¹⁴.

Table 3. Represents the ΔG (kcal/mol) Binding Strength of the developed hydrogels

System	Representation in	Target	ΔG (kcal/m	Binding
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	Docking	Protein	ΔG (kcal/mol)	Strength
Rebaudioside A	Pure ligand	TGF-β1	−9.27	Strong
Rebaudioside A	Pure ligand	SOX9	−9.38	Strong
Rebaudioside A	Pure ligand	HIF-1α	−8.09	Strong
Rebaudioside A	Pure ligand	BMP-2	−8.59	Strong
Carbopol /Agar	Partial ligand	TGF-β1	−7.16	Moderate–Strong
Carbopol /Agar	Partial ligand	SOX9	−7.13	Moderate–Strong
Carbopol /Agar	Partial ligand	HIF-1α	−6.48	Moderate
Carbopol /Agar	Partial ligand	BMP-2	−6.80	Moderate
Rebaudioside-loaded Hydrogel	Composite ligand model	SOX9	−10.49	Strong
Rebaudioside-loaded Hydrogel	Composite ligand model	HIF-1α	−6.77	Moderate
Rebaudioside-loaded Hydrogel	Composite ligand model	TGF-β1	−7.74	Moderate–Strong
Rebaudioside-loaded Hydrogel	Composite ligand model	BMP-2	−7.77	Moderate–Strong
Component	Type	Function in System	Docking Role	
Rebaudioside A	Bioactive compound	Anti-inflammatory, regenerative	Active binding molecule	
Carbopol 940	Synthetic polymer	Gel formation, viscosity control	Drug carrier	
Agar	Natural polymer	Structural scaffold	Drug carrier	
Rebaudioside-loaded	Composite system	Sustained drug	Indirect (release-mediated)	

Hydrogel		release	interaction)
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Table 4. Role of Each Component in the Hydrogel System

System	Ligand Complexity	ΔG Range (kcal/mol)	Interpretation
Pure Rebaudioside A	Fully optimized	−8.0 to −9.4	Strong binding
Partial ligand	Fragmented	−6.5 to −7.0	Reduced binding
Rebaudioside-loaded Hydrogel	Composite structure	−6.7 to −7.7	Moderate binding

Table 5. Effect of Hydrogel Incorporation on Binding Affinity

Parameter	Rebaudioside A Alone	Hydrogel System
Binding affinity	High (−9 range)	Moderate (−7 range)
Stability at target site	Short-term	Long-term sustained
Drug release	Immediate	Controlled release
Bioavailability	Limited	Enhanced
Therapeutic effect	Strong but transient	Sustained and prolonged

Table 6. Mechanistic Contribution of Hydrogel vs Free Drug

Protein	Pathway	Rebaudioside A (ΔG)	Hydrogel System (ΔG)	Overall Effect
BMP-2	Differentiation	−8.59	−7.77	Moderate
TGF-β1	ECM synthesis	−9.27	−7.74	Strong
SOX9	Gene regulation	−9.38	−10.49	Strong
HIF-1α	Hypoxia response	−8.09	−6.77	Moderate–Strong

Table 7. Multi-Target Interaction Profile of Hydrogel System

System	Hydrogen Bond	Hydrophobic	Interaction
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	Residue s	Residues	Strength
Rebaudioside A	Ser88, Asn102, Lys35	Leu90, Val63, Ile62	Moderate
Carbopol/Agar	Not applicable	Not applicable	Carrier only
Rebaudioside-loaded Hydrogel	Ser88, Asn102	Leu90, Val63	Moderate

Table 8. Residue Interaction Profile – BMP-2

System	Hydrogen Bond Residues	Hydrophobic Residues	Interaction Strength
Rebaudioside A	Asn42, Ser49, Lys37, Thr51	Leu45, Val38	Strong
Carbopol/Agar	Not applicable	Not applicable	Carrier only
Rebaudioside-loaded Hydrogel	Asn42, Ser49	Leu45, Val38	Moderate–Strong

Table 9. Residue Interaction Profile – TGF-β1

System	Hydrogen Bond Residues	Hydrophobic Residues	Interaction Strength
Rebaudioside A	Arg75, Ser77, Asn82	Leu80, Val81, Ile76	Strong
Carbopol/Agar	Not applicable	Not applicable	Carrier only
Rebaudioside-loaded Hydrogel	Arg75, Ser77, Asn82, Gln79	Leu80, Val81, Ile76	Very Strong

Table 10. Residue Interaction Profile – SOX9

System	Hydrogen Bond Residues	Hydrophobic Residues	Interaction Strength
Rebaudioside A	Ser247, Asn251, Thr254	Leu248, Val255, Ile259	Strong
Carbopol/Agar	Not	Not	Carrier

gar	applicable	applicable	only
Rebaudioside-loaded Hydrogel	Ser247, Thr254	Leu248, Val255	Moderate

4. CONCLUSION

The present study demonstrates that the Carbopol 940–agar hydrogel system can be effectively engineered to achieve a controlled balance between hydration, structural integrity, and degradation behavior, which are critical parameters for cartilage regeneration. A clear formulation-dependent trend was observed across swelling and degradation confirming that polymer composition plays a decisive role in determining hydrogel performance. The optimized formulation (F5) exhibited superior performance by achieving maximum swelling capacity, controlled degradation. These characteristics are essential for maintaining a hydrated and structurally stable microenvironment conducive to cartilage repair.

Molecular docking studies further revealed that Rebaudioside A possesses strong binding affinity toward key proteins involved in cartilage regeneration, particularly SOX9 and TGF-β1, indicating its potential to modulate chondrogenic pathways. The hydrogel system itself demonstrated moderate interaction, confirming its primary role as a sustained delivery platform rather than a direct bioactive agent.

The integration of physicochemical characterization with molecular docking provides a comprehensive understanding of both material performance and biological interaction. Overall, the developed hydrogel system represents a promising and scientifically robust platform for cartilage regeneration, with potential for further preclinical and translational development.

5. CONFLICT OF INTEREST: None

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