RESEARCH PAPER

Development and Optimization of Stevioside loaded Pluronic F127–Agar Hydrogel for Skin Tissue Regeneration"

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ABSTRACT

This study developed and optimized a Stevioside-loaded Pluronic F127–agar hydrogel for localized drug delivery and skin tissue regeneration. Eight formulations (F1–F8) were prepared by varying agar concentration (0.2–1.6% w/v) while maintaining constant Pluronic F127 (18% % w/v) and Stevioside (100 µg/ml). The hydrogels were evaluated for physicochemical properties, swelling, degradation, water retention, drug loading, in vitro drug release, and morphology. All formulations exhibited acceptable pH (6.31–6.84) and high drug-loading efficiency (94.8–98.8%). Increasing agar concentration improved hydrogel stability and moisture retention but reduced spreadability and drug release. Among the formulations, F5 showed optimal performance with controlled swelling (312 ± 13%), moderate degradation (51.2 ± 1.8%), and sustained drug release over 14 days. Release kinetics followed the Higuchi model with non-Fickian transport. FESEM revealed a porous interconnected microstructure, FTIR confirmed drug–polymer compatibility, and invitro antioxidant potential in 3T3 cell against Lipopolysaccharide demonstrated enhanced cell membrane protection , reduced oxidative stress, and improved antioxidant status . Overall, F5 represents a promising hydrogel platform for sustained wound healing and skin tissue regeneration.

KEYWORDS:hydrogel; Pluronic F127; Agar; Stevioside; Wound healing; Controlled drug release; Skin tissue regeneration; Bio adhesion; Hydrogel optimization.

How to cite this article: Bhimavarapu SK, Shanmugarajan TS. Development and Optimization of Stevioside loaded Pluronic F127–Agar Hydrogel for Skin Tissue Regeneration. Int J Drug Deliv Technol. 2026;16(6): 151-159. DOI: 10.25258/ijddt.16.6.22

Source of support: Nil

Conflict of interest: None

1. INTRODUCTION

Skin tissue regeneration is a complex biological process involving inflammation, cell proliferation, extracellular matrix remodeling, and re-epithelialization. Conventional wound dressings often fail to provide a sustained therapeutic environment, particularly in chronic wounds where prolonged inflammation and impaired tissue repair delay healing. Hydrogels have emerged as promising wound-healing platforms due to their ability to maintain a moist environment, facilitate gas exchange, absorb exudates, and support cellular migration.[1]

Pluronic F127 (Poloxamer 407), a triblock copolymer of polyethylene oxide–polypropylene oxide–polyethylene oxide, is widely used in drug delivery systems because of its reversible sol–gel transition behavior. However, its relatively poor mechanical strength and rapid erosion limit its application in prolonged drug delivery and tissue engineering. To overcome these limitations, natural polymers such as agar can be incorporated to enhance network stability, mechanical integrity, and water-retention capacity.

Stevioside, a diterpene glycoside isolated from Stevia rebaudiana, possesses anti-inflammatory, antioxidant, and wound-healing activities. Nevertheless, its therapeutic effectiveness is limited by rapid diffusion and inadequate retention at the application site. Therefore, a hydrogel-based delivery system capable of sustained and localized stevioside release may improve its regenerative potential.[3]

The present study aimed to formulate and optimize a stevioside-loaded Pluronic F127–agar hydrogel by varying agar concentration to modulate its physicochemical properties, degradation behavior, drug-release profile, and biological performance. The objective was to develop a structurally stable and biocompatible hydrogel capable of providing sustained drug delivery and an optimal microenvironment for effective skin tissue regeneration.

2. METHODOLOGY

2.1 Preparation of Stevioside loaded Hydrogel

Stevioside-loaded Pluronic F127/Agar hydrogels were prepared using a combination of the cold method and thermal dispersion technique. Agar was dissolved in distilled water at 85–90°C with continuous stirring and maintained at 45–50°C to prevent premature gelation. Pluronic F127 was gradually dispersed in chilled

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distilled water (4°C) and refrigerated for 12–24 h to ensure complete hydration. Stevioside was dissolved in distilled water and incorporated into the Pluronic solution, followed by the addition of warm agar solution under continuous stirring. The resulting homogeneous hydrogel was stored at 4°C until further characterization.[4]

Table 1. Composition of Stevioside-loaded Pluronic F127/Agar Hydrogels

Formulation	Pluronic F127 (% w/v)	Agar (% w/v)	Stevioside (% w/v)
F1	18	0.2	100 µg/ml
F2	18	0.4	100 µg/ml
F3	18	0.6	100 µg/ml
F4	18	0.8	100 µg/ml
F5	18	1.0	100 µg/ml
F6	18	1.2	100 µg/ml
F7	18	1.4	100 µg/ml
F8	18	1.6	100 µg/ml

2.2 Swelling Behavior

Swelling behavior was assayed by placing pre-weighed samples of hydrogel in phosphate-buffered saline (PBS, pH 7.4) at 37 C. At specific time points, the swollen hydrogel was taken out, blotted to remove excess liquid on the surface and then weighted. The equation to compute swelling ratio was:

Swelling Ratio (%) = [(Wt − W0) / W0] × 100

Where: W0 = the weight of dry or pre-weighed hydrogel at the beginning of the experiment.

• Wt = weight of swollen hydrogel at time t Swelling is a very crucial parameter of skin regenerative hydrogel since a perfect wound-healing matrix needs to take up exudate, be hydrated, yet retain structure without overly disintegrating.[4]

2.3 Adhesiveness / Bioadhesion

Adhesiveness was measured by putting a specified amount of hydrogel between two clean glass slides or a model biological membrane and a constant load within a pre-determined time. The amount of force needed to separate the two surfaces was measured and tabulated. This experiment was an empirical determination of the capacity of the hydrogel to stick to wet skin or wound. In skin tissue regeneration, moderate-high adhesiveness is preferable since it enhances retention of sites, wound coverage, and local drug retention time.[5]

2.4 Texture Assessment

Manual compression between fingers, and flat-surface compression of each hydrogel to estimate the firmness, cohesiveness, elasticity, and structural recovery was assessed. This is a useful qualitative test used in the screening of formulations used to heal wounds because the gel used should have adequate softness to allow tissue integration but be firm enough to stay in place once applied.[6]

2.5 Degradation Study

The amount of hydrogel (W0) was known, and it was immersed in PBS (pH 7.4) and allowed to incubate at 37 o C. The hydrogel samples were removed, gently blotted, dried to a constant weight and weighed (Wt) at predetermined intervals. The equation was used to determine percentage degradation:

Degradation (%) = [(W0 − Wt) / W0] × 100

One of the desirable qualities of skin tissue regeneration hydrogels is controlled degradation; the material must last long enough to allow local tissue healing and sustained delivery of drugs, but not to last too long or disrupt normal tissue remodelling.

2.6 Water Retention Capacity

The retention capacity of water was calculated by placing pre-swollen hydrogel samples in ambient or controlled conditions, and recording the weight of water that was retained in the sample as time-lapsed. The equation used to determine water retention was:

Water Retention (%) = (Wt / W0) × 100

This parameter is very applicable in wound care since an effective hydrogel that retains water can help to retain a moist healing environment, which is advantageous to epidermal migration, collagen remodelling and decreased crust formation.[7]

2.7 Release Kinetics

The in vitro drug-release data of the stevioside-loaded hydrogels were fitted to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas kinetic models to elucidate the release mechanism. The respective equations used were $Qt = Q0 + k0t$, $\log Qr = \log Q0 - k1t/2.303$, $Qt = kHt1/2$, and $Mt/M\infty = kKPtn$. Linear regression analysis was performed, and the coefficient of determination (R^2) was used to identify the best-fit model. The release exponent (n) obtained from the Korsmeyer–Peppas model was used to characterize the drug-release mechanism as Fickian diffusion, anomalous transport, or polymer relaxation-controlled release.[8]

2.8 Field Emission Scanning Electron Microscopy (FESEM)

FESEM was used to assess the morphology and interior porous structure of the optimum Stevioside-loaded Pluronic F127/Agar hydrogel. Hydrogels are highly hydrated and can easily collapse under vacuum, therefore in order to maintain their three-dimensional microstructure, the samples were initially freeze-dried (lyophilized). The lyophilized samples of hydrogel were broken to reveal the inner cross-section and fixed

to the aluminum stubs through conductive adhesive tapes. The sputter-coated samples were mounted and a thin sheet of gold or platinum was sprayed over the samples to eliminate charging when imaging. FESEM micrographs were taken on various magnifications to observe the roughness of the surface, pore size, pore connectivity, internal channels and compactness of the structure of the hydrogel matrix. The microstructural properties of the Stevioside-containing hydrogel were contrasted with the control hydrogel to determine the effect of drug addition on the inner structure of the formulation.[9]

2.9 Fourier Transform Infrared Spectrophotometry (FTIR)

The analysis conducted through FTIR was employed to identify the compatibility of the chemical and any potential intermolecular interaction between Stevioside, Pluronic F127 and agar. FTIR spectra of pure Stevioside, pure Pluronic F127, pure agar, blank hydrogel, and optimized drug-loaded hydrogel were taken. All samples were dry enough then analyzed to reduce interference due to water. The spectra were measured between 4000-400cm-1 in the wavenumber region of the range (KBr pellet method or ATR mode). The characteristic peaks related to the major functional groups of the drug and polymers were determined and compared. The optimized hydrogel spectrum was observed in terms of peak shifts, peak broadening, loss of characteristic peaks, or emergence of new peaks, which may be an indication of drug-polymer interaction or incompatibility.[10 , 11]

2.10 *In vitro* antioxidant of stevioside-loaded hydrogel on Lipopolysaccharide (LPS)- induced 3T3 cell lines

LPS as an oxidative-stress inducer and Matrix degradation model To investigate LPS induced oxidative stress , Sterile blank and Stevioside-loaded hydrogel were added directly within 96-well plates. 3T3 mouse fibroblasts were seeded onto the hydrogel matrices at a high density of 1 X 105 cells/mL to ensure precise surface attachment. After 24 hours of acclimatisation, inflammation was induced by replacing the culture media with fresh complete DMEM containing 10 µg/mL Lipopolysaccharide (LPS**)**. Following a 24-hour incubation, supernatants were collected to quantify Glutathione and Lipid Peroxidation were estimated [12 , 13]

3. Results and Discussion

3.1 Swelling Behavior

The swelling behavior of the hydrogels showed a non-linear trend, increasing from formulations F1 to F5 and decreasing from F6 to F8. At low agar concentrations, the matrix lacked sufficient structural support for maximum water uptake. Moderate agar levels improved the balance between network integrity and free volume, enhancing swelling while maintaining coherence. However, higher agar concentrations produced a denser polymer network that restricted pore expansion and fluid penetration, reducing swelling capacity. Appropriate swelling is essential for wound healing, as hydrogels must absorb exudate while remaining hydrated and mechanically stable. Among all formulations, F5 (312 ± 13%) exhibited the most balanced and optimal swelling behavior for wound-healing applications.

3.2 Adhesiveness and Texture

The further agar concentration augmented adhesiveness and texture firmness, which suggests augmented cohesiveness of matrices and elevated interfacial interaction. Recipes of higher agar concentration were more resistant to deformation and had a higher retention between contact surfaces. This trend is advantageous to wound-healing hydrogels up to a given extent. Moderate adhesiveness is used to ensure that the hydrogel stays in place at the wound site to minimize displacement and enhance local drug residence. Nevertheless, too much adhesiveness and high firmness can decrease conformability and result in the hydrogel being too stiff or hard to spread in a unified manner. Thus, F7 and F8 were found to be the most adhesive and firm, but less practical than F5 which allowed a large site-retention potential without becoming un-bending.

3.3 Degradation Study

The degradation study showed an inverse relationship between agar concentration and mass loss, with low-agar formulations degrading rapidly and high-agar formulations exhibiting slower, controlled degradation. F1 showed the highest degradation, while F8 remained largely intact due to its denser polymer network. For wound healing, balanced degradation is essential to provide temporary support without hindering tissue remodeling. Among all formulations, F5 displayed the most favourable profile, maintaining initial structural integrity while gradually degrading over time, making it suitable for skin tissue regeneration applications.

3.4 Water Retention analysis

Water-retention studies showed that increasing agar concentration improved the hydrogel's ability to maintain internal hydration due to enhanced water-binding capacity and network stability. Higher agar formulations retained moisture longer, which is beneficial for wound healing by promoting epithelialization, collagen remodeling, and patient comfort. However, optimal performance depends on a balance of properties rather than water retention alone. F5 demonstrated excellent moisture retention without the excessive compactness or handling limitations associated with higher agar concentrations, making it the most suitable formulation.

Table 2. Physico chemical characterisation of the developed hydrogel scaffolds

Formulation	Swelling (%) ± SD	Degradation (%) ± SD	Retention (%) ± SD

F1	210 ± 9	78.2 ± 2.5	62.4 ± 2.3
F2	238 ± 10	71.6 ± 2.2	66.8 ± 2.1
F3	265 ± 11	65.3 ± 2.0	71.2 ± 2.0
F4	289 ± 12	58.7 ± 1.9	75.9 ± 1.8
F5	312 ± 13	51.2 ± 1.8	80.3 ± 1.7
F6	285 ± 11	44.6 ± 1.7	83.6 ± 1.6
F7	254 ± 10	38.9 ± 1.5	86.9 ± 1.5
F8	228 ± 9	33.4 ± 1.4	89.2 ± 1.4

3.5 Kinetic Modeling of Drug Release

The release data were fitted to various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, and the corresponding parameters are summarized in the following figure (Figure 1 , Figure2, Figure 3).The Higuchi model showed the highest correlation coefficient (R^2 = 0.8618), indicating that drug release is predominantly governed by diffusion through the hydrated polymer matrix. The slope value further supports the diffusion-controlled mechanism.

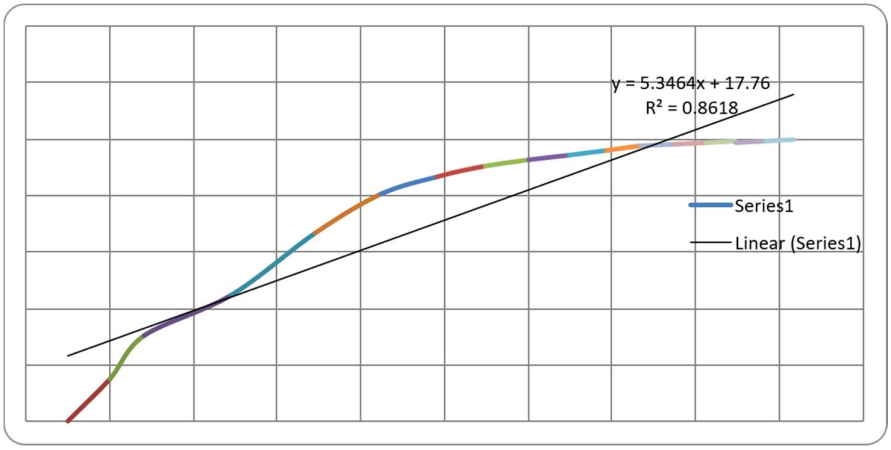

Figure 1. Zero Order plot of the optimised F5 Formulation

The zero-order model exhibited a lower correlation (R^2 = 0.6693), suggesting that the release is not constant over time. Similarly, the first-order model showed moderate fitting (R^2 =0.3662), indicating partial dependence on drug concentration.

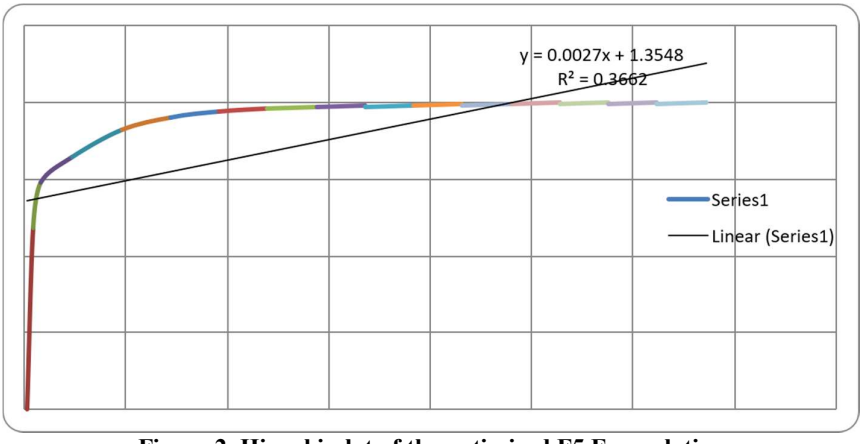

Figure 2. Higuchi plot of the optimised F5 Formulation

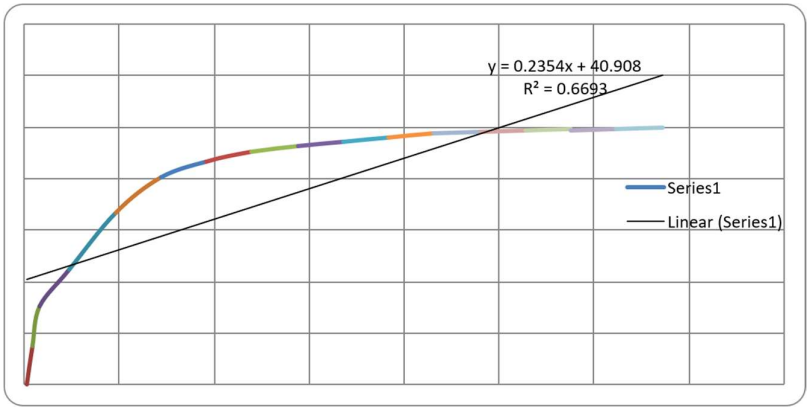

Figure 3. Korsmeyer–Peppas plot of the optimised F5 Formulation

The Korsmeyer–Peppas model provided significant insight into the release mechanism, with a release exponent indicating non-Fickian (anomalous) transport, where both diffusion and polymer relaxation contribute to drug release. The high correlation coefficient (R^2 = 0.3925) confirms the suitability of this model for describing the release behavior.

3.6 Field Emission Scanning Electron Microscopy (FESEM)

FESEM analysis of the optimized stevioside-loaded Pluronic F127/Agar hydrogel (F5) revealed a highly porous, interconnected, sponge-like microstructure favorable for wound healing and skin tissue regeneration. The well-distributed pores facilitate fluid penetration, nutrient diffusion, gas exchange, and drug transport while maintaining a moist wound environment. Compared to the blank hydrogel, F5 exhibited a slightly denser structure, indicating drug incorporation. The absence of crystalline drug deposits confirmed uniform stevioside distribution, supporting its sustained-release behavior and suitability for tissue regeneration applications.

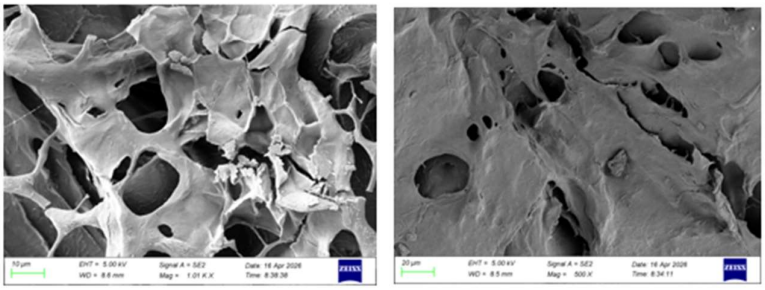

Figure 4. FESEM of the Pluronic F127/Agar Hydrogel

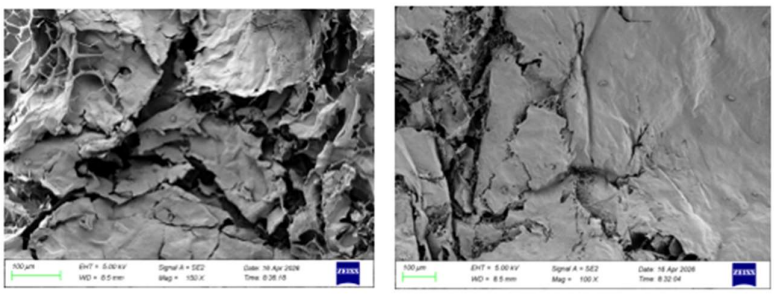

Figure 5. FESEM of the Stevioside loaded Pluronic F127/Agar Hydrogel

3.7 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of the Pluronic F127–Agar hydrogel confirmed the presence of characteristic functional groups from both components, indicating successful composite hydrogel formation. A broad peak at 3425 cm^{-1} corresponded to O–H stretching, suggesting extensive hydrogen bonding between agar and Pluronic F127. The peak at 2887 cm^{-1} represented aliphatic C–H stretching, while the band at 1643 cm^{-1}

indicated absorbed water, reflecting the hydrogel’s water retention ability. Peaks at 1348 cm^{-1} and 1106 cm^{-1} were attributed to C–H bending and C–O–C stretching vibrations, respectively. The FTIR spectrum of the stevioside-loaded hydrogel further confirmed successful drug incorporation within the hydrogel matrix.

The FTIR spectrum of the Stevioside-loaded Pluronic F127–Agar hydrogel showed characteristic peaks confirming successful drug incorporation and interaction with the hydrogel matrix. A broad peak at 3419 cm^{-1} corresponded to O–H stretching vibrations, indicating enhanced hydrogen bonding due to the hydroxyl groups of stevioside. The peak at 2892 cm^{-1} was assigned to aliphatic C–H stretching, while the band at 1641 cm^{-1} represented H–O–H bending of absorbed water, reflecting the hydrogel’s water retention capacity. Peaks at 1456 cm^{-1} and 1103 cm^{-1} corresponded to C–H bending and C–O–C stretching vibrations, respectively, confirming the coexistence of Pluronic F127, agar, and stevioside within the hydrogel network. The FTIR spectrum of the optimized stevioside-loaded hydrogel (F5) retained all characteristic peaks with only minor shifts and broadening, indicating hydrogen bonding and physical interactions between stevioside and the polymer matrix. No new or missing peaks were observed, confirming drug–polymer compatibility and formulation stability.

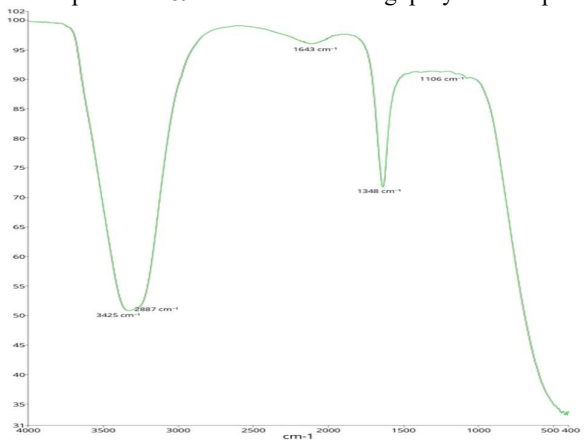

Figure 6. FTIR Spectra of the Pluronic F127/Agar Hydrogel

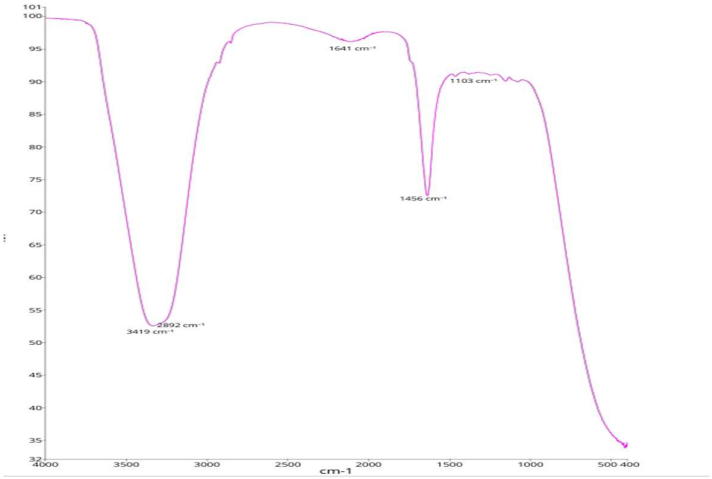

Figure 7. FTIR Spectra of the Stevioside loaded Pluronic F127/Agar Hydrogel

3.8 *In vitro* antioxidant of stevioside-loaded hydrogel on Lipopolysaccharide (LPS)-induced 3T3 cell lines

3T3 under normal control conditions and those exposed to LPS -induced oxidative stress were evaluated to assess the protective effects of hydrogel formulations. Cells treated with oxidative stress showed a reduction in intracellular antioxidant levels, particularly glutathione and increased MDA . Application of a blank hydrogel provided minimal improvement in cellular redox balance. In contrast, treatment with a Stevioside -loaded hydrogel significantly enhanced glutathione levels in stressed 3T3 Cells. This suggests that Stevioside delivery via hydrogel effectively mitigates oxidative damage and restores antioxidant capacity. Overall, the Stevioside -loaded hydrogel demonstrates promising potential as a therapeutic strategy for protecting skin cells against oxidative stress–induced damage.

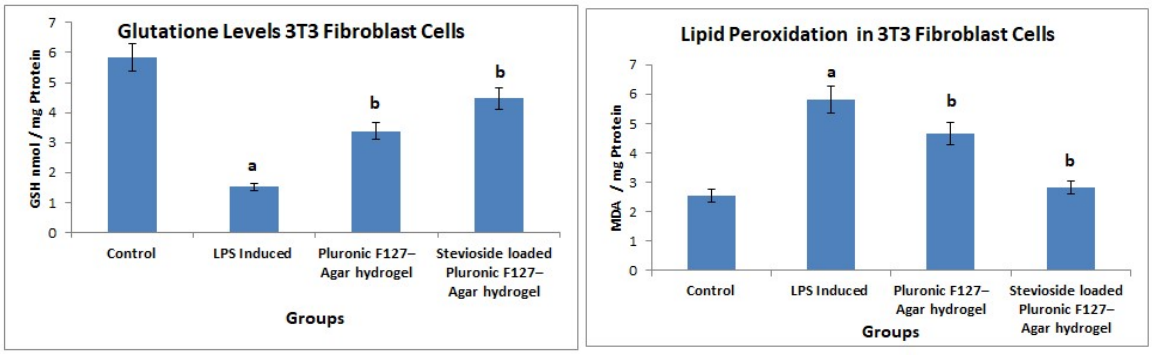

Fig- 8 In vitro Glutathione and Lipid-Peroxidation levels of stevioside-loaded hydrogel on Lipopolysaccharide (LPS)-induced 3T3 cell lines.

Levels of GSH and LPO in the Control and of the experimental Groups . Comparisons are made between: a- Control and LPS induced ; b-Group LPS Induced and Pluronic F127/Agar Hydrogel, Stevioside loaded Pluronic F127/Agar Hydrogel .a, b, Statistically significant ($p<0.05$);

4. CONCLUSION

The study demonstrated that agar concentration significantly affects the structural, physicochemical, and drug-release properties of Pluronic F127 hydrogels. Among all formulations, F5 was optimized, exhibiting controlled swelling, moderate degradation, high drug content, and sustained drug release suitable for wound healing. Drug release followed the Higuchi model with non-Fickian transport. SEM revealed a porous interconnected structure, FTIR confirmed drug–polymer compatibility, Stevioside -loaded hydrogel significantly enhanced glutathione levels in stressed 3T3 Cells showed protection and regeneration. Overall, F5 represents a promising hydrogel system for localized drug delivery and skin tissue regeneration.

5. CONFLICT OF INTEREST: None

6. ACKNOWLEDGEMENT: The authors thank the Vels Institute of Science, Technology and Advanced Studies for the facility extended.

7. REFERENCES

1. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010r;89(3):219-29. doi: 10.1177/0022034509359125.
2. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res. 2006;23(12):2709-28. doi: 10.1007/s11095-006-9104-4.
3. Tonogaki K, Itami K, Yoshida J. Catalytic four-component assembly based on allenylboronate platform: new access to privileged allylic amine structures. J Am Chem Soc. 2006;128(5):1464-5. doi: 10.1021/ja057778a.
4. Wei F, Zhu H, Li N, Yu C, Song Z, Wang S, Sun Y, Zheng L, Wang G, Huang Y, Bao Y, Sun L. Stevioside Activates AMPK to Suppress Inflammation in Macrophages and Protects Mice from LPS-Induced Lethal Shock. Molecules. 2021;26(4):858. doi: 10.3390/molecules26040858.
5. Panthi, Vijay Kumar, Jha, Saurav Kumar, Pangeni, Rudra, Paudel, Keshav Raj, Formulation and Development of Adapalene Topical Nanohydrogel Using Different Surfactants and Cosurfactants for Antiacne Activity: In Vitro and Ex Vivo Evaluation, Journal of Nanomaterials, 2022, 6889293, 19 pages, 2022. https://doi.org/10.1155/2022/6889293
6. Al-Barghouthy EY, Hamed S, Mehyar GF, AlKhatib HS. Comparative Evaluation of Spreadability Measurement Methods for Topical Semisolid Formulations/A Scoping Review. Gels. 2025;11(12):1006. doi: 10.3390/gels11121006.
7. Zustiak SP, Leach JB. Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. Biomacromolecules. 2010;11(5):1348-57. doi: 10.1021/bm100137q.
8. AL-Smadi, K., Ali, M., Zhu, J. *et al.* Advances in Characterization of Transdermal and Topical Products using Texture Analyzer Systems. *AAPS PharmSciTech* 26, 157 (2025). https://doi.org/10.1208/s12249-025-03148-x
9. D'Souza SS, DeLuca PP. Development of a dialysis in vitro release method for biodegradable microspheres. AAPS PharmSciTech. 2005;6(2):E323-8. doi: 10.1208/pt060242.
10. Sun Y, Ku BJ, Moon MJ. Microstructure of the silk fibroin-based hydrogel scaffolds derived from the orb-web spider Trichonephila clavata. Appl Microsc. 2024;54(1):3. doi: 10.1186/s42649-024-00096-x.
11. Am Ende MT, Peppas NA. Analysis of drug distribution in hydrogels using fourier transform infrared microscopy. Pharm Res. 1995;12(12):2030-5. doi: 10.1023/a:1016224813626.
12. Persinal-Medina M, Llames S, Chacón M, Vázquez N, Pevida M, Alcalde I, Alonso-Alonso S, Martínez-López LM, Merayo-Lloves J, Meana Á. Polymerizable Skin Hydrogel for Full Thickness Wound Healing. Int J Mol Sci. 2022; 23(9):4837. doi: 10.3390/ijms23094837.

13. Al-Asmari AK, Al-Saeed AH, Al-Saeed MS. Attenuation of lipopolysaccharide-induced oxidative stress and inflammation by natural antioxidants in human dermal fibroblasts. J Cell Biochem. 2021;122(3):345–356. doi: 10.1002/jcb.29863.