Development, Physico Chemical Characterization and in-Vitro Studies of Pluronic F127/ Agar Hydrogel Containing Stevioside for Topical Application

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Abstract

Hydrogels are macromolecules that are naturally occurring and have unique properties like biocompatibility, cell-controlled degradability, and intrinsic cellular interaction that eventually affect their use in tissue engineering, notably for the development of new tissues. Most of the time, hydrogels facilitate cell migration, angiogenesis, high water content, and enhanced transportation of nutrients. Because of their biochemical resemblance to the skin extracellular matrix, scaffolds have drawn attention and are now recognised as a unique tool for skin tissue engineering concepts. In this study, a Stevioside loaded Pluronic F127/ Agar hydrogel scaffold was designed using the physical cross-linking technique that mirrored the structure and composition of skin extracellular matrix. To achieve the study's primary goal of a composite scaffold that degrades at a controlled

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1. Introduction

A highly structured, hypo cellular, and flexible tissue is skin tissue. It has long been established that skin tissue defects, which are typically brought on by trauma, tumours, or degenerative pathologies, have a very low inherent capacity for self-healing. The clinical therapy of skin tissue defects is still incredibly difficult, nevertheless. Traditional clinical therapies like autografts and allografts have had some success but have drawbacks like complicated procedures and mishaps post-operative complications, high cost, and immune-related issues.[1]

Hydrogels are macromolecules that are naturally occurring and have unique properties like biocompatibility, cell-controlled degradability, and intrinsic cellular interaction that eventually affect their use in tissue engineering, notably for the development of new tissues. Most of the time, hydrogels facilitate cell migration, angiogenesis, high water content, and enhanced transportation of nutrients. Because of their biochemical resemblance to the skin extracellular matrix, scaffolds have drawn attention and are now recognised as a unique tool for skin tissue engineering concepts.[2]

Pluronics or Poloxamers are FDA-approved non-toxic poly(ethylene oxide), poly(propylene oxide), and poly(ethylene oxide) triblock copolymers (PEO-PPO-PEO). These aqueous solutions go through a sol-to-gel transition when the temperature is raised over an LCGT. The molecular weight of the constituent parts and the proportion of hydrophobic to hydrophilic units vary amongst the different types of Pluronics produced. Consequently, Pluronics enable the creation of thermosensitive hydrogels with various properties, such as critical gelation concentration (CGC) and gelation time at physiological conditions. Pluronic F127 (F127) gels have received much attention in the literature as cell and drug transporters because of their low toxicity, reverse thermal gelation, high drug loading efficacy, and potential to gel in physiological conditions at relatively low concentrations.[3]

Agar is a mixture of polysaccharides from various seaweeds belonging to the Rhodophyceae (red algae) class, including Gelidium and Gracilaria, which stand in for the major structural elements in the cell walls. Due to its superior gelling characteristics, or the fact that it produces gels with comparatively high temperature stability and gel strength, this substance is frequently employed in the field of skin tissue engineering concepts.[4]

The substance that is most prevalent in Stevia rebaudianaBertoni leaves is stevioside. Many nations, including Brazil, Korea, Japan, and the United States, have formally approved stevioside to be used as food additives and nutritional supplements, respectively. Due to its anti-hypertensive, anti-oxidative, anti-tumor promoting, anti-glycaemic, anti-hypertensive, anti-oxidative, anti-oxidative, and anti-inflammatory properties, stevioside has been reported to have numerous health benefits for humans.[5]

In this study, a Stevioside loaded Pluronic F127/ Agar hydrogel scaffold was designed using the physical cross-linking technique that mirrored the structure and composition of skin extracellular matrix. To achieve the study's primary goal of a composite scaffold that degrades at a controlled rate.

2. Materials & Methods

Materials

Stevioside and Pluronic F127 were obtained from Tokyo Chemical India Pvt. Ltd (Chennai, India). Agar-Agar powder was obtained from HI-media. All the samples we used in this study were obtained from genuine sources and were used as such.

Methodology

Preparation of the Stevioside loaded hydrogel scaffolds

Initially the Pluronic F127 and Agar - Agar at predefined concentrations (%w/v) were dissolved in the deionized water at 6°C to avoid micellisation and/or gelation during solution preparation. Once clear solution was obtained Stevioside was added to the above polymeric solution. Finally the mixture allowed to stabilise at the temperature of 37 ^{o}C for 48 hours to form the hydrogel scaffolds.

Organoleptic properties

Hydrogel compositions have been visually assessed for appearance, color, and texture. When assessing the grittiness of hydrogels,

it was once done by rubbing a little amount of gel between the thumb and index finger to see if there were any coarse particles present.[6]

Viscosity:

Using a Brookfield cup and bob viscometer with spindle number S64 (model-LVDVE, Brookfield Engineering Laboratories, MA, USA), the viscosities of all hydrogel formulations were measured at a temperature of 25°±2°C. At 10 RPM, each measurement was made in triplicate, and the mean value was calculated.[7]

pH:

A digital pH metre (Mettler- Toledo India Private Limited, Powai Mumbai, India) that had been calibrated with buffered solutions of various pH levels was used to measure the formulation's pH at 25° ± 2°C. 10 ml of distilled water were used to dissolve 1 g of hydrogel, which was weighed precisely. Each hydrogel's pH was measured in triplicate, and the average value was determined.[8]

Spreadability:

One method of assessing the spreadability of hydrogels was to measure the spreadability diameter of 1g of hydrogel between two glass plates. To ensure that the gel spreads uniformly between the plates, 1g of weight was placed in between the plates for 1 minute.[9]The following equation was originally used to determine the gel spreadability:

S=ML/T

Where S=spreadability, M=weight tied to higher slide, L=length of glass slide and T=time taken by way of the slide to separate from.

***Invitro* release**

Dialysis bags were filled with a specific quantity of Stevioside loaded hydrogels, which were then incubated with constant agitation in 20 mL of PBS (pH = 6.3). At predetermined intervals, 2 mL of buffer was removed and swapped out for an equal volume of new media. The absorbance was measured at 360nm by using the UV-vis spectrophotometry and the invitro drug release graph was plotted. Three independent replicates were used to compute the mean and standard deviation at the predefined time intervals.[9]

Cell Viability Assay

On the NIH-3T3 cells, cell viability studies were evaluated using the MTT assay. The reduction of yellow 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase, which only takes place in metabolically active cells, was examined in the study that was conducted. In a nutshell, cells were seeded in 96-well plates at a density of 1 x 10^4 cells/well and maintained in DMEM with 10% bovine calf serum (BCS), 1% penicillin/streptomycin, and 37 °C with 5% CO_2. The medium was then withdrawn and a new one was added that included various concentrations of HyDrO-DiAb (45-100 µg/ml). Cell viability was assessed 24 hours after incubation by adding 10 µl of MTT solution to each well. 100 µl of DMSO for formazan crystal solubilization was added after 1 hour of incubation at 37 °C in a 5% CO_2 environment. The absorbance of decreased MTT was measured at 360 nm in a microplate reader after 10 min of incubation in the dark (Synergy h1 Hybrid reader Biotek). Comparing cell viability to control wells, it was represented as a percentage.[10]

3. Results And Discussion

Organoleptic Characteristics

The bodily characteristics of the Stevioside loaded hydrogel scaffold, such as appearance, colour, and homogeneity, have been assessed. Both the physical appearance and the silky texture of each gel composition were once appropriate (as shown in table 1). However, choosing the excellence of a method by solely relying on its organoleptic properties used to hardly ever be difficult. Hence due to this reason the other characterisation studies

were also performed for the developed hydrogel.

Table 1. Depicts the organoleptic properties of the developed hydrogel scaffolds

S. No	Organoleptic properties	Pluronic F 127/Agar hydrogel	Stevioside loaded hydrogel
1.	**Colour**	Clear and transparent	Pale yellow
2.	**Consistency**	Very gentle Semisolid	Very gentle Semisolid
3.	**Homogeneity**	Homogenous	Homogenous

pH

The topical formulations' pH should be suitable with the skin's pH to prevent irritation or damage of the skin. Stevioside loaded hydrogel scaffold' pH values fall within the permissible range for topical administration, allowing for the usage of the developed hydrogel.

Viscosity

The rheological characteristics of topical hydrogel formulations may significantly affect the Spreadability, adhesiveness, drug release, and subsequent penetration while delivering the drug molecules onto or across the skin. The formulation's viscosity results show that the hydrogels that have been produced have enough consistency to stay on the skin. The findings showed that hydrogel formulation viscosities were constant.For the Pluronic F127/ Agar hydrogel and Stevioside loaded hydrogel formulas, the values of the viscosity were 12284±325.03cP and 13837±681.47 cP, respectively.

Spreadability

Spreadability refers to a gel's capacity to cover a surface after being applied to the skin's surface. This is a crucial component of topical formulation for improved patient adherence and consistent gel application to the skin's surface. The spreadability of more viscous formulations is poor, which makes application challenging. The kind and quantity of gelling agent employed in the formulation has an impact on it. All formulations with good spreadability exhibit uniform spreading and spread more quickly when applied. Results of developed hydrogels' spreadability were shown in a table2**.**

Table 2: Represents the pH, Viscosity and Spreadability of the developed formulations

S. No	Characterisation study	Pluronic F 127/Agar hydrogel	Stevioside loaded hydrogel
1	pH	5.91±0.021	6.24±0.017
2	Viscosity	12284±325.03cP	13837±681.47cP
3	Spreadability	7.25±0.34 (g.cm/s)	6.81±0.29 (g.cm/s)

***Invitro*release**

The diffusion and disintegration of the hydrogel's backbone served as the primary mediators for the therapeutic chemicals' release. While long-term release of Stevioside successfully promoted cell migration, proliferation, and ECM formation in later stages, which were ideally timed with the process of wound healing, the burst release of stevioside in the early phase may timely decrease inflammation and oxidative stress. In the current study, the Pluronic F127/Agar hydrogel released stevioside in a noticeable burst, with a cumulative release percentage of up to 4.2 ± 0.71 % initially and 75.5 ± 1.26% on day 14 (Figure 1) . Further the stevioside from the hydrogels continued to be delivered continuously for an additional three days after the release rate was reduced down.

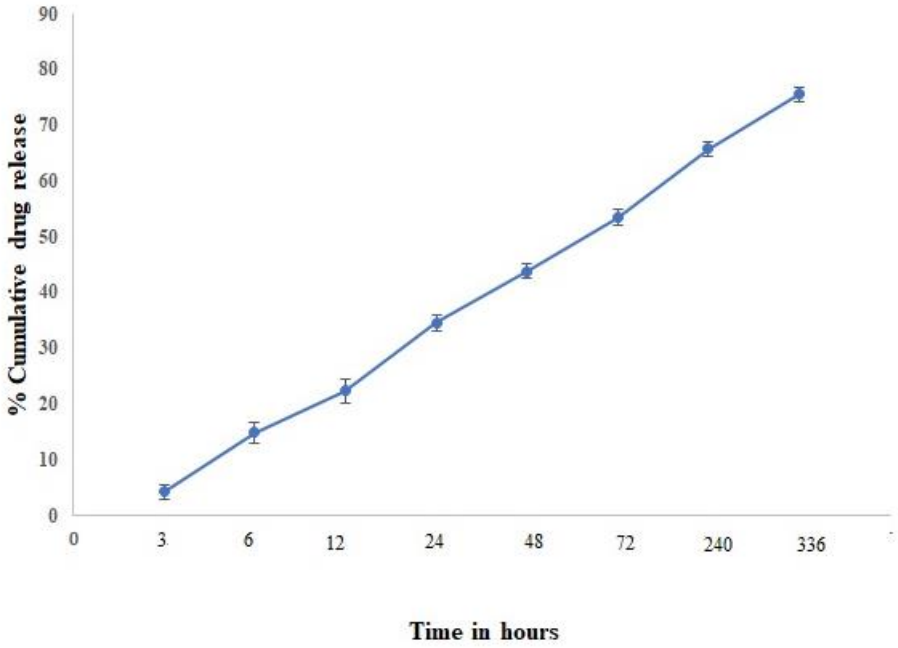

Figure 1. Represents the Invitro release profile of the Stevioside loaded Pluronic F127/Agar hydrogel.

Cell Viability Assay

The hydrogels' cytotoxicity was assessed 24 and 72 hours after cell seeding using the MTT assay kit. The findings show that the produced hydrogels are not only cytocompatible but also have an impact on cell proliferation. Stevioside beneficial impact on NIH-3T3 cells development at both incubation durations is demonstrated in figure 2.In comparison to Control group at 24 hours, the cell proliferation on the Stevioside loaded Pluronic F127/Agar hydrogel is statistically significant. After 72 hours of cell seeding, it is even much higher than Pluronic F127/Agar hydrogel. These findings imply that the addition of Stevioside to the Pluronic F127/Agar hydrogel has improved their suitability for cell proliferation due to its ideal impact on cell growth (Figure 2).

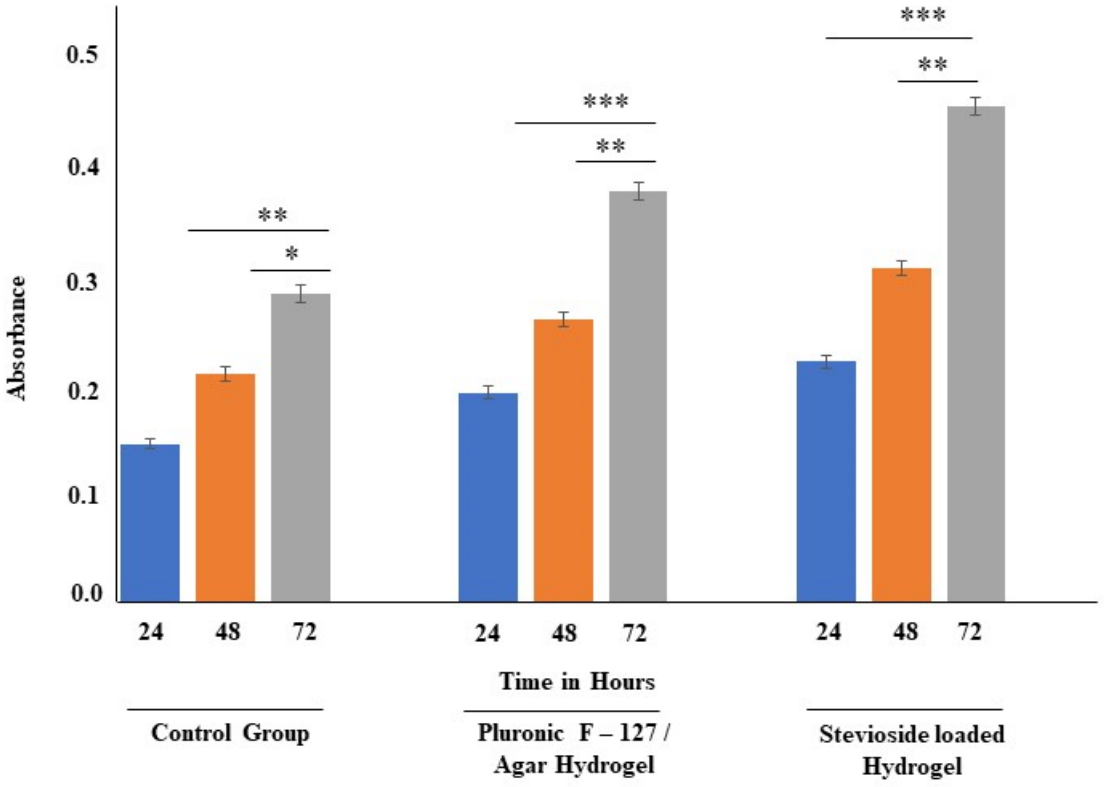

Figure 2. Depicts the Cell viability of the Control group, Pluronic F127/Agar Polymeric hydrogel and Stevioside loaded hydrogel.

4. Conclusion

This work assessed the impact of Stevioside loaded Pluronic F127/Agar hydrogel containing Stevioside on the tissue engineering studies. Further the characterisation studies demonstrated the ideal mechanical characteristics of the developed hydrogels. Furthermore, the cells in the Stevioside loaded Pluronic F127/Agar hydrogel showed higher cell proliferation rates than the control group, according to the cell viability studies. Finally, the results of this study imply that fabricated Stevioside loaded Pluronic F127/Agar hydrogel -based wound dressings hold promise for effective wound care.

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