Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

Development, characterization, and cytotoxicity of starch-based electrospun nanofibrous scaffold incorporated with bioactive compound from *Cayratia trifolia*

Bhuvaneswari Meganathan[1], Mani Panagal[2]*

[1,2*]Department of Biotechnology, Annai College of Arts and Science (Affiliated by Bharathidasan University), Kovilacheri, Tamil Nadu 612503, India.

***Corresponding author:** Mani Panagal

(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS

Cayratia trifolia; Linolenyl alcohol; Electrospinning; Starch-based nanofibrous scaffolds; Biomedical applications

ABSTRACT:

This study focuses on developing starch-based nanofibrous scaffolds encapsulated with linolenyl alcohol, a bioactive compound from *Cayratia trifolia*. Electrospun nanofibers were created by blending linolenyl alcohol with starch solutions at varying concentrations (10%, 20%, and 40% v/v) and extensively characterized. Scanning electron microscopy revealed nanofiber diameters between 73 and 95 nm for those encapsulating linolenyl alcohol, and this exhibited superior thermal stability compared to free linolenyl alcohol, as confirmed by thermogravimetric analysis. Fourier-transform infrared (FT-IR) spectroscopy affirmed the interaction between starch and linolenyl alcohol within the nanofibers, confirming successful encapsulation. This incorporation not only maintained the compound's integrity but also enhanced its controlled release potential, rendering it promising for drug delivery applications. The application of 40% linolenyl alcohol-loaded nanofibers resulted in a noteworthy increase in the viability of HaCat cells. These findings suggest that these scaffolds hold potential as versatile platforms for tissue engineering and controlled drug release, thereby advancing innovative biomedical applications. This research contributes to the burgeoning fields of biomaterials and biopharmaceuticals, highlighting the significance of natural compounds in scaffold design for advancing medical therapies.

Introduction

In the ever-evolving landscape of biomaterials and biopharmaceuticals, the development of innovative platforms for tissue engineering and controlled drug release is a constant pursuit. One such avenue of exploration lies in the utilization of electrospun nanofibrous scaffolds, which have garnered significant attention in recent years due to their unique combination of structural versatility, biocompatibility, and controlled release capabilities [1]. Tissue engineering is a rapidly advancing field that seeks to address the growing demand for effective solutions in regenerative medicine and wound healing. It encompasses the design and construction of artificial tissues and organs, aiming to replace or repair damaged biological tissues in the human body. Central to the success of tissue engineering is the concept of scaffolds, which serve as a three-dimensional template to support cell growth, tissue formation, and ultimately the restoration of normal physiological function. These scaffolds are tasked with mimicking the extracellular matrix (ECM) – the natural structural framework of tissues – to facilitate cell adhesion, proliferation, and differentiation [2]. The design of an ideal scaffold is a multifaceted challenge. It must be biocompatible, providing a supportive environment for cell attachment and growth. Additionally, it should possess a porous structure to enable the exchange of nutrients and waste products, and its mechanical properties should match those of the target tissue. However, achieving all of these characteristics in a single scaffold can be complex [3].

In recent years, electrospun nanofibers have emerged as a promising solution to many of the challenges in scaffold design. The electrospinning process involves the extrusion of a polymer solution or melt from a fine nozzle under the influence of an electric field. The charged polymer solution forms an electrified jet that is subsequently stretched and collected on a grounded collector, resulting in the formation of ultrafine fibers with diameters in the nanometer range [4]. These nanofibers inherently possess a high surface area-to-

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

volume ratio and an interconnected porous structure that closely mimics the ECM, making them an ideal candidate for tissue engineering applications. The versatility of electrospinning allows the use of a wide range of materials, both synthetic and natural, to create nanofibrous scaffolds [5]. These materials can be tailored to meet specific requirements, and among them, starch stands out as a biodegradable and biocompatible natural polymer. It is derived from renewable sources, making it an eco-friendly option in the realm of biomaterials. This starch-based approach offers a sustainable solution to the growing demand for regenerative therapies [6].

While the mechanical and structural properties of the scaffold are crucial, a comprehensive approach to tissue engineering should also consider the bioactive component. Beyond serving as a structural support, scaffolds can be designed to deliver bioactive compounds that enhance the healing and regenerative processes. These compounds can include growth factors, vitamins, or even natural plant extracts. *Cayratia trifolia*, a plant found in various regions, is known for its rich pharmacological properties [7]. Among the bioactive compounds extracted from *Cayratia trifolia*, linolenyl alcohol holds particular promise. It is a bioactive compound with demonstrated therapeutic potential, making it an intriguing candidate for incorporation into electrospun nanofibers [8]. Linolenyl alcohol is known for its antioxidant, anti-inflammatory, and wound healing properties. Integrating such a bioactive compound into the scaffold not only offers structural support but also the potential to enhance tissue regeneration and wound healing [9].

In this context, the present research manuscript delves into the development and characterization of starch-based electrospun nanofibrous scaffolds that are ingeniously incorporated with linolenyl alcohol, a bioactive compound derived from the ethanolic extracts of *Cayratia trifolia*.

2. Materials and Methods

2.1. Preparation of Polymeric Solutions for Electrospinning

Polymeric solutions were prepared using soluble potato starch in a solvent mixture of formic acid and deionized water. Initially, 5 g of starch was dissolved in 10 mL of formic acid and deionized water. To achieve starch gelatinization, the solutions were stirred for 24 hours and aged for an additional 24 hours. Different concentrations of linolenyl alcohol, a bioactive compound previously isolated from an ethanolic extract of Cayratia trifolia by our research group, were incorporated into the starch solution in dry bases, with concentrations of 0%, 10%, 20%, and 40% (v/v). After adding linolenyl alcohol, the solutions were stirred for 15 minutes before the electrospinning process. For the control group, no linolenyl alcohol was added. To ensure complete polymer dissolution, the polymeric solutions were sealed and allowed to age for 24 hours at room temperature before the electrospinning process.

2.2. Solution Characterization

The viscosity and conductivity of the four prepared solutions prior to electrospinning were determined using a Brookfield Model DV-II + Pro Viscometer and a Metrohm-914 Conductometer.

2.3. Electrospinning Process

The electrospinning process was conducted using a syringe infusion pump (KD Scientific, Model 100, Holliston, England) and an electrospinning apparatus. The fiber-forming solution was loaded into a 3 mL plastic syringe equipped with a stainless-steel spinneret needle with a 0.7 mm diameter. The distance between the spinneret and the grounded stainless-steel collector plate was set at 20 cm. Electrospun nanofibers were produced at room temperature (25 °C ± 2 °C) with a relative humidity maintained at 45% (± 2%) using a dehumidifier. A steady flow rate of 0.60 mL/h was maintained, and an applied voltage of +25.0 kV and –3.0 kV was used during the electrospinning process. To eliminate impurities and maintain the nanofibrous sheets at 25 °C for drying, the sheets were rinsed three times with water and phosphate buffer solution (PBS).

2.4. Physicochemical Characterization of Fabricated Nanofibers

2.4.1. Morphology and Size Distribution of the Nanofibers

To assess the morphology and size distribution of the nanofibers, a scanning electron microscope (SEM) (Jeol, JSM-6610LV, USA) was employed. Samples were sputter-coated with a thin layer of gold and analyzed at an acceleration voltage of 10 kV. SEM micrographs were captured, and the size distribution and average diameter of fifty randomly selected nanofibers were determined using ImageJ software (version 2015).

2.4.2. Nanofibers' Thermal Stability

The thermal stability of the fabricated nanofibers was assessed using a thermogravimetric analyzer (TGA) (TA-60WS, Shimadzu, Kyoto, Japan). Approximately 5 mg of the nanofiber samples were placed in platinum capsules, and the analysis was conducted with a heating rate of 10 °C per minute in a temperature range of 30–600 °C, under a nitrogen flow of 50 mL per minute. An empty platinum capsule was used as a reference.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

2.4.3. Fourier-Transform Infrared (FT-IR) Spectrum of Nanofibers

The infrared spectrum of the fabricated nanofibers and their individual constituents, namely soluble potato starch and linolenyl alcohol, was obtained using an attenuated total reflection (ATR) accessory (IR-affinity, Shimadzu, Japan). The analysis involved 120 scans at a spectral resolution of 4 cm within a wavenumber range of 4000–500 cm, conducted at room temperature (25 °C ± 2 °C).

2.5. Cytotoxicity analysis

In accordance with the ISO 10993-5 standard test method, an essential evaluation of the electrospun nanofibrous mats' in vitro cytotoxicity was conducted. This assay plays a pivotal role in ensuring the safety and biocompatibility of the fabricated nanofibers, a critical consideration for their potential use in biomedical applications. To prepare the specimens for this assessment, they were subjected to a sterilization process by immersion in 70% ethanol for 30 minutes. Subsequently, the specimens were washed with phosphate-buffered saline (PBS) under sterile conditions. This rigorous sterilization process is essential to prevent any potential contamination and to ensure the integrity of the cell culture. HaCat cells, serving as a well-established model for human keratinocytes, were then cultured in 96-well plates.

The cell density was precisely controlled, with 5×10^3 cells per well, and they were nurtured in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS) and 1% antibiotics, consisting of 100 U/mL of penicillin and 100 g/mL of streptomycin. The culture plates were maintained at a temperature of 37 °C in a controlled environment with 5% carbon dioxide (CO2) for a duration of 24 hours. To gauge the viability and proliferation of the cultured cells, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed. This widely recognized colorimetric assay is based on the conversion of MTT to formazan crystals by metabolically active cells. The assay allows for the quantitative determination of cell viability and proliferation. The optical density (OD) of the formed formazan crystals was measured at a wavelength of 540 nm using an ELISA Reader, specifically the ELX 808. This optical density detection provides a reliable and quantifiable measure of the cellular activity and vitality.

2.5. Statistical Analysis

The data are reported as the mean values along with the corresponding standard deviations (SD) to provide a comprehensive overview of the dataset. To evaluate the significance of differences between the two groups under consideration, a statistical t-test was applied. A p-value threshold of less than 0.05 was used as the criterion for statistical significance, indicating that results with a p-value below this threshold were deemed as statistically significant. This statistical analysis was conducted to ascertain the presence and magnitude of variations or disparities between the compared groups, ensuring the reliability and validity of the obtained results.

3. Results and Discussion

3.1. Solution Characterization

In the preparation of polymeric solutions for the electrospinning process, it is essential to characterize certain properties of these solutions, such as viscosity and conductivity, which can significantly impact the subsequent electrospinning results. As detailed in Table 1, various polymeric solutions were meticulously prepared with distinct compositions, specifically varying in their linolenyl alcohol content. Figure 1 and Figure 2 illustrate the variations in viscosity and conductivity among these diverse solutions. An important observation from these results is that solutions with a higher percentage of linolenyl alcohol (C and D) exhibited notably elevated levels of both viscosity and conductivity. This observation can be attributed to the complex interplay between the constituents of the solutions.

Increased viscosity, as observed in solutions with higher linolenyl alcohol content, is often a consequence of enhanced intermolecular and intramolecular hydrogen bonding between the starch and linolenyl alcohol components. This heightened interaction between the constituents reduces surface tension, a critical factor in electrospinning, and contributes to the formation of more uniform and structured nanofibers. The intricate interplay of these molecular forces fosters a favorable environment for the electrospinning process. Moreover, the inclusion of starch, which contains approximately 25% amylase, further influences the viscosity of the solutions. Amylase, a component of starch, is a long linear polymer consisting of 1,4-glucopyranoside units. Its interaction with linolenyl alcohol adds another layer of complexity to the solution's viscosity, with amylase potentially forming chains and entangling with linolenyl alcohol molecules. This characterization of the polymeric solutions is pivotal in understanding and predicting the electrospinning outcomes, as it sheds light on the dynamic relationships between the components within the solutions. By fine-tuning these properties, researchers can optimize the electrospinning process to yield nanofibers with tailored characteristics for various applications in fields

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

like tissue engineering and controlled drug delivery [10].

3.2. Physicochemical Characterization of Fabricated Nanofibers

3.2.1. Morphology and Size Distribution

The fabricated nanofibers displayed diverse morphologies and sizes, as evident in Figure 3. Notably, the nanofibers lacking linolenyl alcohol exhibited a ribbon-shaped and fused morphology. This unique structure can be attributed to the absence of stretching during the electrospinning process. In contrast, nanofibers containing linolenyl alcohol exhibited a distinct morphology characterized by homogeneity, cylindrical shape, and random orientation. This suggests that the solvent used in the process evaporated more rapidly in the presence of linolenyl alcohol. The average diameters of these nanofibers were measured at 96 nm, 72 nm, 83 nm, and 94 nm for varying linolenyl alcohol concentrations (0%, 10%, 20%, and 40%, respectively).

The successful achievement of these homogeneous, bead-less nanofibers with diameters ranging from 72 nm to 96 nm was a result of adjustments made to the solution parameters and electrospinning conditions. Notably, the high starch concentration (50% w/v) used in this study did not yield high viscosity in the formic acid solvent. Intriguingly, the introduction of linolenyl alcohol into the solution rendered it more amenable to the electrospinning process. Recent studies have reported the production of ultrafine fibers from soluble potato starch, but these exhibited beaded-fiber morphologies with average diameters ranging from 128 to 143 nanometers. The modifications made in this study aimed to produce bead-less nanofibers with controlled diameters of 72 to 96 nm. This innovation opens the possibility of utilizing these nanofibers in applications related to wound healing and controlled release of linolenyl alcohol [11].

3.2.2. Thermal Stability of the Nanofibers

The thermal stability of the nanofibers was assessed through thermogravimetric analysis (TG), as depicted in Figure 4. It was observed that nanofibers lacking linolenyl alcohol (0%) exhibited similar weight loss behavior to pure soluble potato starch. This included moisture evaporation at approximately 100 °C, followed by starch decomposition occurring in the temperature range of 250–300 °C. In contrast, free linolenyl alcohol, with a boiling point of approximately 374 °C, displayed a one-step weight loss pattern at 160 °C.

Nanofibers containing linolenyl alcohol (10%, 20%, and 40%) exhibited a more complex weight loss pattern with three distinct stages. The first stage involved moisture evaporation at around 100 °C. The second stage, occurring between 200 and 250 °C, was likely attributed to the degradation of linolenyl alcohol. The third stage, ranging from 250 to 300 °C, witnessed the decomposition of starch. These findings confirm the successful encapsulation of linolenyl alcohol within the starch fibers and suggest that these nanofibers possess enhanced thermal stability compared to the free compound [12].

3.2.3. Fourier-Transform Infrared (FT-IR) Spectroscopy Analysis of the Nanofibers

The FT-IR spectra presented in Figure 5 provide insights into the chemical composition of the nanofibers and their individual constituents. Characteristic bands attributed to the stretching vibrations of inter- and intra-molecular bound hydroxyl groups in soluble potato starch were observed. Specifically, the bands at 2941 cm^{-1} correspond to the stretching of C-H bonds, while those at 1641 cm^{-1} are indicative of water content in the starch. The bands at 1351 cm^{-1} represent C-O-H bending or CH2 deformation, and the bands at 1153 and 1020 cm^{-1} relate to the stretching of single bonds and double bonds. The band at approximately 920 cm^{-1} likely corresponds to the glycosidic bonds present in glucose.

Distinct functional groups characteristic of linolenyl alcohol were also evident in the FT-IR spectra. An absorption band at 1728 cm^{-1} signified the presence of a carbonyl group, while a shoulder peak at 2858 cm^{-1} indicated an aldehyde hydrogen group (CHO). Additionally, bands at 2924 cm^{-1} corresponded to C-H stretching, and those at 1458 and 1381 cm^{-1} were associated with C-H bending bonds. The presence of a C=C group was suggested by the band at 1529 cm^{-1}.

These FT-IR spectroscopy results underscore the unique chemical composition of the nanofibers, which combine the characteristics of soluble potato starch and linolenyl alcohol [13]. This information is vital in elucidating the chemical interactions within the nanofibers, contributing to our understanding of their potential applications in various fields, particularly in the context of controlled release and wound healing [14].

3.3. Cytotoxicity analysis

In the realm of wound care and tissue engineering, the quest for the ideal wound dressing or scaffold material is characterized by one paramount criterion—compatibility. The ability of a material to seamlessly integrate with the biological milieu is a hallmark of its potential for success in the intricate processes of tissue regeneration and wound healing. To ascertain this critical attribute, an MTT test was conducted on the electrospun nanofibrous mats, offering valuable

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

insights into their biocompatibility when exposed to Human Keratinocytes (HaCat Cells) over a span of 24 and 48 hours. Figure 6, a visual representation of the MTT test results, provides a pivotal view of the electrospun nanofibrous mats' interaction with HaCat cells. The MTT assay is a gold standard in biomedical research, enabling the quantitative assessment of cell viability, a fundamental parameter in evaluating the biocompatibility of materials [15].

The findings of this test are significant, as they underscore the ability of the electrospun nanofibrous mats to sustain cell viability and compatibility [16]. After 24 hours of incubation with HaCat cells, the nanofibrous mats exhibited a remarkable cell viability ranging from 100% to 130%, with the control sample serving as the baseline (100%). The results, therefore, indicate that the mats not only did not harm the cells but also, in some cases, promoted their growth and metabolic activity. These findings affirm that the electrospun nanofibrous mats, crafted with meticulous precision, hold the potential to serve as exceptional candidates for tissue engineering applications [17]. Their biocompatibility, as evidenced by the MTT test results, positions them as materials that not only avoid harm to surrounding cells but actively support their growth and metabolic activity [18]. In the context of wound care and tissue regeneration, such attributes are of paramount importance, as they contribute to the overall success of these materials in promoting the healing and restoration of damaged tissues.

Tables

Table 1: . Prepared polymeric solution composition and weight ratio

S. No.	Samples	Weight ratio (%)	
		Starch	Linoleny alcohol
1.	A	100	0
2.	B	90	10
3.	C	80	20
4.	D	60	40

Figures

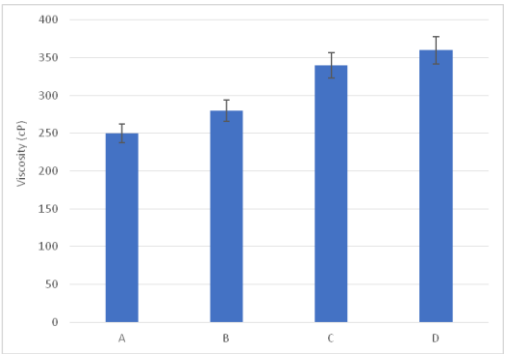

Figure 1: It provides valuable insights into the viscosity of the fabricated nanofibers.

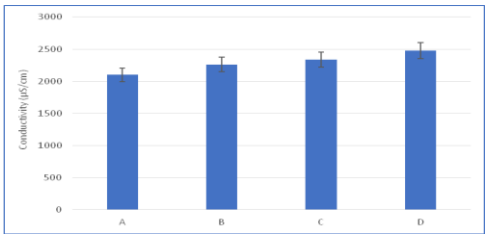

Figure 2: It illuminating glimpse into the conductivity of the fabricated nanofibers.

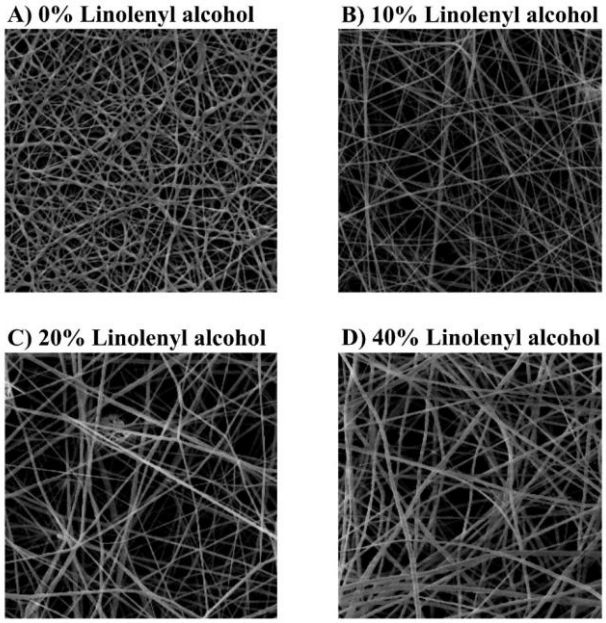

Figure 3: This figure offers a captivating visual exploration of the morphological characteristics of the fabricated starch nanofibers that have been enriched with linolenyl alcohol.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

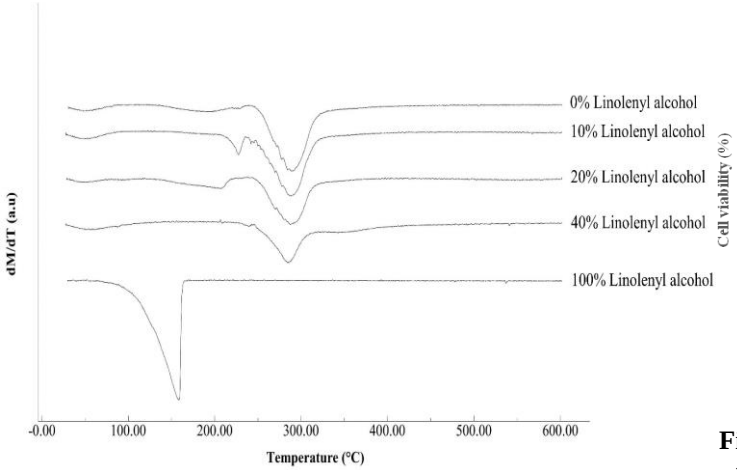

Figure 4: It provides a comprehensive insight into the thermal stability of the fabricated starch nanofibers that have been infused with linolenyl alcohol.

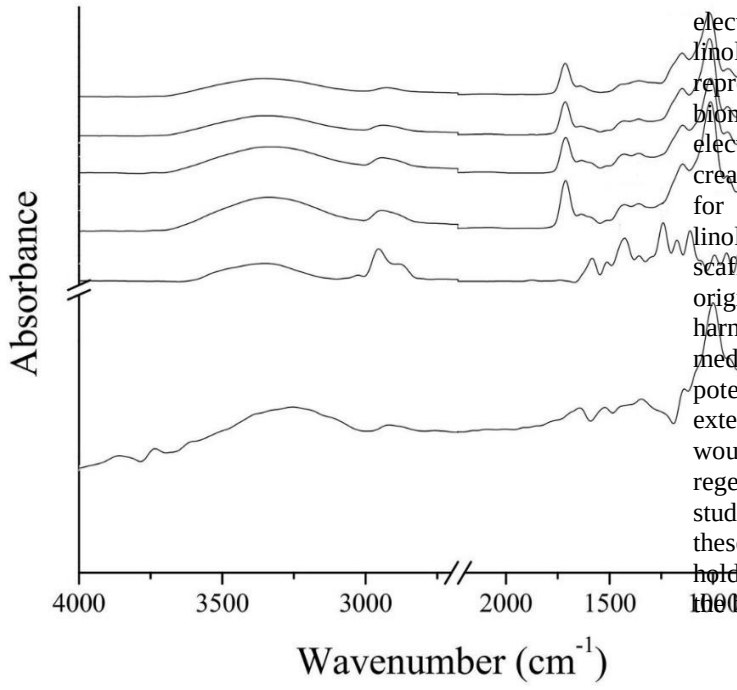

Figure 5: It provides a comprehensive depiction of the Fourier-Transform Infrared (FT-IR) analysis conducted on the fabricated starch nanofibers enriched with linolenyl alcohol.

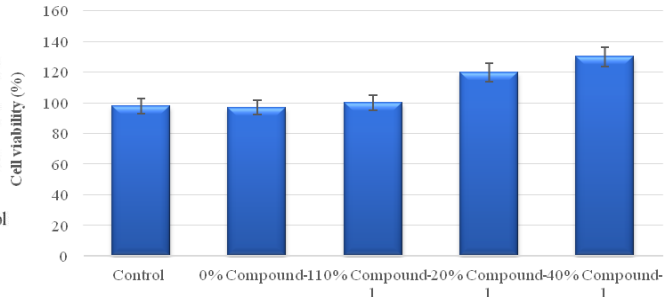

Figure 6: It showcases the results of a critical *in vitro* assay that investigates the impact of the fabricated nanofibers on the viability of HaCat cells.

4. Conclusion

The development and characterization of starch-based electrospun nanofibrous scaffolds incorporated with linolenyl alcohol derived from *Cayratia trifolia* represent an exciting frontier in the fields of biomaterials and biopharmaceuticals. The use of electrospun nanofibers offers a unique opportunity to create scaffolds with the desired structural properties for tissue engineering, while the incorporation of linolenyl alcohol adds a therapeutic dimension to these scaffolds, making them multifunctional. The natural origins of the materials emphasize the importance of harnessing nature's resources for the advancement of medical therapies and regenerative medicine. The potential applications of these innovative scaffolds extend to various biomedical contexts, including wound healing, controlled drug delivery, and regenerative medicine. As we delve deeper into this study, we will uncover the intricacies of developing these promising scaffolds and the implications they hold for advancing the field of biomedical research and the broader landscape of healthcare.

5. Acknowledgement: We authors would like to express our sincere gratitude to the Department of Biotechnology, Annai College of Arts and Science, Kumbakonam, Tamil Nadu, India for completing this research work in such a fine manner.

6. References

1. Palanisamy C.P., Alugoju P., Jayaraman S., Poompradub, S., 2023. *Nigella sativa* L. seed extracts promote wound healing progress by activating VEGF and PDGF signaling pathways:

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4s), 364-370 | ISSN:2251-6727

An *in vitro* and *in silico* study. F1000Research. 12, 436.

2. Pei, J., Palanisamy, C. P., Alugoju, P., Anthikapalli, N. V. A., Natarajan, P. M., Umapathy, V. R., Swamikannu, B., Jayaraman, S., Rajagopal, P. and Poompradub, S. (2023). A Comprehensive Review on Bio-Based Materials for Chronic Diabetic Wounds. Molecules, 28(2), 604.
3. Xu, Z., Han, S., Gu, Z. and Wu, J. (2020). Advances and impact of antioxidant hydrogel in chronic wound healing. Advanced healthcare materials, 9(5), 1901502.
4. Pei, J., Prasad, M., Mohamed Helal, G., El-Sherbiny, M., Abdelmonem Elsherbini, D. M., Rajagopal, P., Palanisamy, C. P., Veeraraghavan, V. P., Jayaraman, S. and Surapaneni, K. M. (2022). Beta-Sitosterol Facilitates GLUT4 Vesicle Fusion on the Plasma Membrane via the Activation of Rab/IRAP/Munc 18 Signaling Pathways in Diabetic Gastrocnemius Muscle of Adult Male Rats. Bioinorganic Chemistry and Applications, 2022: 7772305.
5. Stino, A. M., Rumora, A. E., Kim, B., Feldman, E. L. (2020). Evolving concepts on the role of dyslipidemia, bioenergetics, and inflammation in the pathogenesis and treatment of diabetic peripheral neuropathy. Jounrla of the Peripheral Nervous System, 25(2), 76-84.
6. Anjum, S., Rahman, F., Pandey, P., Arya, D. K., Alam, M., Rajinikanth, P. S., Ao, Q. (2022). Electrospun Biomimetic Nanofibrous Scaffolds: A Promising Prospect for Bone Tissue Engineering and Regenerative Medicine. International Journal of Molecular Sciences, 23(16), 9206.
7. Palanisamy, C. P., Cui, B., Zhang, H., Jayaraman, S., Muthukaliannan, G. K. *(2020).* A comprehensive review on corn starch-based nanomaterials: Properties, simulations, and applications. Polymers, 12(9), 2161.
8. Palanisamy, C. P., Cui, B., Zhang, H., Gunasekaran, V. P., Ariyo, A. L., Jayaraman, S., Rajagopal, P. and Long, Q. (2022). A critical review on starch-based electrospun nanofibrous scaffolds for wound healing application. International Journal of Biological Macromolecules, 222 (Part B): 1852-1860.
9. Perumal, P. C., Sowmya, S., Pratibha, P., Vidya, B., Anusooriya, P., Starlin, T. and Gopalakrishnan, V. K. (2014). Identification of novel PPARγ agonist from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.): a computational molecular simulation studies. Journal of Applied Pharmaceutical Science, 4(9), 6-11.
10. Sowmya, S., Perumal, P. C., Ravi, S., Anusooriya, P., Shanmughavel, P., Murugesh, E., Chaithanya, K. K. and Gopalakrishnan, V. K. (2021). 1-Pentacosanol Isolated from Stem Ethanolic Extract of *Cayratia trifolia* (L.) is A Potential Target for Prostate Cancer-In SILICO Approach. Jordan Journal of Biological Sciences, 14(2).
11. Perumal, P. C., Sowmya, S., Velmurugan, D., Sivaraman, T. and Gopalakrishnan, V. K. (2016). Assessment of dual inhibitory activity of epifriedelanol isolated from *Cayratia trifolia* against ovarian cancer. Bangladesh Journal of Pharmacology, 11(2), 545-551
12. Sowmya, S., Perumal, P. C., Anusooriya, P., Vidya, B., Pratibha, P.and Gopalakrishnan, V. K. (2015). *In vitro* antioxidant activity, in vivo skin irritation studies and HPTLC analysis of *Cayratia trifolia* (L.) Domin. International Journal of Toxicological and Pharmacological Research, 7(1), 1-9.
13. Meganathan, B., Palanisamy, C. P. and Panagal, M. (2021). Antioxidant, antimicrobial and cytotoxicity potential of n-hexane extract of *Cayratia trifolia* L. Bioinformation, 17(3), 452.
14. Palanisamy, C. P., Pethanan, S., Vincent, G. G., Murugesan, K., Ramachandran, A., Sivanandam, R. and Panagal, M. (2021). Chemotherapeutic potential of *Cayratia trifolia* L nhexane extract on A2780 cells. Bioinformation, 17(8), 710.
15. Perumal, P. C., Reddy, C. P. K., Pratibha, P., Sowmya, S., Priyanga, S., Devaki, K., Ramkumar, S. and Gopalakrishnan, V. K. (2015). CXCR4 inhibitory activity analysis of linoleic acid isolated from ethanolic extract of *Cayratia trifolia* (L.): an molecular docking simulation. International Journal of Pharmacognosy and Phytochemical Research, 7(4), 781-784.
16. Kong, L., Ziegler, G. R. (2013). Quantitative relationship between electrospinning parameters and starch fiber diameter. Carbohydrate Polymers, 92(2), 1416-1422.
17. Fonseca, L.M., Silva, F.T., Antunes, M.D., El Halal, S.L.M., Lim, L., Dias, A.R.G. (2019). Aging time of soluble potato starch solutions for ultrafine fibers formation by electrospinning. Starch - Stärke 71, 1–7.
18. Bao, P., Kodra, A., Tomic-Canic, M., Golinko, M. S., Ehrlich, H. P., Brem, H. (2009). The Role of Vascular Endothelial Growth Factor in Wound Healing. Journal of Surgical Research, 153(2), 347–358.