UW Journal of Science and Technology Vol. 9 (2025)
ISSN: 2523-0123 (Print) 2616-4396 (Online)

Aloe Vera and Curcumin-Infused Zein Nanofibers for Diabetic Foot Ulcer

Nisha Iqbal[1 & 2], Haris Tejani[1], Aisha Siddiqa[1], Heer Memon[1], Abdul Qadir Ansari[1], Rabia Almas Arain[1]

[1]Mehran University of Engineering and Technology Jamshoro, Sindh, Pakistan.

[2]Department of Bio-Medical Engineering, University of Naples Federico II, Italy.

Corresponding Author: engr.nishaiqbal@gmail.com

DOI: 10.56220/uwjst.v9i.238

Abstract

One of the most prevalent diseases in the world, diabetes has a high death and comorbidity rate in addition to complications like diabetic foot ulcers (DFU). According to numerous clinical reports, the infection, alterations in inflammatory responses, lack of extracellular matrix, and angiogenesis failure are the main causes of the difficulties in healing the DFU. Natural material-replicating nanofibers have high porosity, superior moisture absorption, and a higher rate of oxygen exchange. For the therapy of DFU, these qualities are very desirable. Using a biodegradable polymer called Zein, we produced a nanofibrous scaffold in this study that contained two natural medicinal ingredients: curcumin and Aloe Vera. Using the SEM, FTIR, and UV-VIS characterization methods, respectively, the morphology of the nanofibrous scaffold was analyzed, confirming that the fibers were precisely the right size and had a smooth, bead-free structure; the presence of chemical components (C-O, C=C and S=O, and C=N groups of Cr and Av with Zein polymer) was also assessed, and the pattern of drug release was found to be effective along with excellent antibacterial performance.

Keywords: *Scaffold, Nanofibers, Diabetic Foot Ulcers, and DFU*

1. Introduction

Diabetic Foot Ulcer (DFU) is a persistent condition that affects the big toes and can damage the foot all the way to the bones [1]. According to reports, the inability to properly close a wound caused DFU is usually associated with wound infections, abnormal responses to inflammation, an imbalance in extracellular matrix (ECM), and angiogenesis failure. [2]. DFU is one of the most common and a highly vulnerable complication in diabetic patients [3] There will be over 360 million diabetes individuals by 2030 [4]. Diabetic foot ulceration affects 4–10% of diabetic person; elderly patients are more likely to develop the illness. Individuals with diabetes have an approximate 15% lifetime risk of developing this condition, and it is estimated that 5% of all diabetic individuals have a history of foot ulcers. About 60–80% of ulcer heal, yet 5- 24% eventually lead to limb amputation while 10-15% continue to be active [5].

There are a number of contributing causes to diabetic foot ulcer (DFU). According to research, people who have a history of foot ulcers or amputation, foot pressure, peripheral edema, low socioeconomic status, plantar calluses, ischemia, kidney disease, and retinopathy, as well as those who are older and have chronic diabetes, have a significantly higher risk of developing DFU. Health care and education are reportedly two major risk factors for foot ulcers [6].

Wound dressings are currently expected to protect wound and hasten the healing activity. Traditional dressings, such as gauze, cotton, pads, and bandages, are widely used in medical settings because of their low cost and simple manufacturing process. However, they are not the best choice for treating wounds due to a number of functional constraints, such as their inability to maintain a moist environment and their permeability to the nutrients needed for proper tissue growth [7]. The development of contemporary dressings during recent decades has revolutionized DFU therapy [8]. The best dressings must keep the area wet, exhibit antibacterial action, promote growth factors, permit oxygen and nutrient exchange, eliminate exudates, and play an essential part in the granulation of tissues. They should also encourage autolytic debridement and re-epithelialization. Additionally, it must be extremely effective, consistent in duration of action, and have a regulated release of medications at the wound site. [9][10].Because of these requirements, no wound dressing satisfied all of the requirements for a DFU wound. Selection of dressing is closely related to the effects of foot ulcer, the site of the wound, the severity, the size of the lesion, the exudation, the infection, the pain, the need for adhesives, and compatibility [11].

Many studies concentrated on dressings for DFU. It has been demonstrated that the electrospinning technology may produce nanofibrous mats that contain a variety of therapeutic and antibacterial substances. A study was conducted to compare the effects of Nigella sativa oil gel and Aloe vera gel on wound healing in diabetic rats. In this research work, Aloe vera shows the best DFU wound healing properties. The created nanofibers were effective in DFU wound healing; in vitro and in vivo testing was performed. The testing shows that AV is showing more antimicrobial activity and effective wound healing as compared to Nigella sativa [12]. The aloe vera plant possesses anti-inflammatory, antibacterial, and antifungal qualities, as well as hypoglycemic benefits [13]. Another study identified curcumin as a potential novel treatment for skin-related disorders [14].The crystalline substance curcumin, also known as diferuloylmethane, is what gives the East Asian spice turmeric its vivid yellow hue. Although turmeric has been used in alternative medicine for thousands of years, curcumin has not yet become a part of our standard dermatologic therapeutic arsenal [14]. The experimental techniques also covered the in vitro and in vivo studies. The prepared nanocomposites demonstrated sustained release of curcumin at the location of the wound and healing effectiveness in animal studies. Nanocomposites were also advised for future commercialization and clinical testing based on the findings. Combining a medicinal drug with curcumin to induce autolytic debridement, tissue granulation at the infected position may improve the efficacy of the resulting composites [15].

A yellow-organic polyphenol molecule with a low molecular weight, curcumin (diferuloylmethane) is the active component of turmeric, an Asian spice, cosmetic, and therapeutic agent. In recent years, researchers have looked at the potential benefits of curcumin, including its biodegradability, antibacterial, anti-inflammatory, and wound healing qualities. Low oral bioavailability, poor solubility, and limited clinical application have hindered curcumin's ability to be used therapeutically. It is feasible and advantageous to incorporate curcumin into nanofibrous patches to enable its administration with enhanced solubility [16]. Therefore, in our research, we created a nanofibrous scaffold including curcumin and aloe vera, owing to their superior therapeutic efficacy, and encapsulated them in zein, which is a naturally occurring, biocompatible, and biodegradable polymer obtained from corn. Zein is a flexible polymer that may be treated to produce a variety of morphologies, including particles, films, membranes, and scaffolds.

2. Materials and Methods

The following main substances were used in the synthesis of the nanofibrous scaffold: Zein Polymer, Dimethylformamide (DMF), Aloe vera, and Curcumin.

First, we extracted curcumin from the turmeric to begin the manufacture of the nanofibrous scaffold. Turmeric was inspected to make sure the components were clean and free of contaminants prior to extraction. A little piece of turmeric was first dried in an oven set to 40°C and then crushed using an electric grinder. The powder samples were then immersed in ethyl acetate at a 1:10 (w/v%) ratio for 70 hours overnight. The unfiltered extract was then passed three times through nylon meshes. The solvent was removed from the final turmeric extract (curcumin) using heat, and it was then stored at 5 °C for future use [17].

Aloe vera and curcumin-loaded zein nanofibers were synthesized using the electrospinning technique. Zein nanofibers with Aloe Vera and Curcumin encapsulated were created. A 3 g total stock of zein solution was made. 40% (1.2 g) of Zein is dissolved in DMF (dimethylformamide) solvent to create the polymeric solution (1.81 g). Aloe vera was added in at a percentage of 10% and curcumin at a proportion of 5% by weight of zein, and the mixture was mixed for three hours under warming conditions. 3 g of polymeric solution total stock was created. A high voltage power supply (capable of producing voltages up to 100 kV) was employed in an electrospinning arrangement. Zein polymeric solution containing curcumin and aloe vera was injected using 3 ml plastic syringes with capillary tips that had an inner diameter of 0.6 mm and were angled 10° from the horizontal plane. In syringes, a polymeric solution was applied to the copper wire positive electrodes. The collector was grounded to the negative electrode. The voltage was optimized to 15 kV. The optimal tip-to-collector distance (TCD) was kept to be 15 cm. At temperature (27–29 °C), nanofibers (NF) were collected over a collector up to five hours. Aluminium foil was used to collect the nanofiber film, which was then dried for 24 hours outside before being collected.

3. Characterization Techniques

We characterized our nanofiber product using SEM, FTIR spectroscopy, and UV-Vis spectroscopy. SEM was used to determine the morphological structure of nanofibers. Using ImageJ software, the typical diameter of nanofibers was calculated from the SEM images. Through the use of an FTIR spectrometer and ATR-FTIR spectra, the chemical structure was examined. Through the use of a UV-Vis spectrophotometer, the drug pharmacokinetics of the polymeric solution was determined.

3.1. Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) employs a focused electron beam to scan the surface of a sample and produce its images. Signals generated by electron interactions with sample atoms reveal the surface topography and chemical composition of the sample. An image is generated by integrating the position of the electron beam and the strength of the signal received while being scanned in a raster scan pattern. The main components of SEM are the source of the electron beam, lenses on different columns, sample chamber, electron detectors, and a display or computer to view images. An item is swept across by a moving electron beam in a scanning electron microscope; electrons dispersed by the object are concentrated by magnetic "lenses" to create a picture of the object's surface that resembles one on a television. The visuals, which might be of little animals or pieces of them, compounds like DNA, or even enormous individual atoms, appear to be three-dimensional (e.g., uranium, thorium) [18].

3.2.**Fourier transform infrared spectroscopy**

FTIR is a technique used to measure the absorption or emission of infrared light by solids, liquids, or gases. An FTIR spectrometer captures high-resolution spectral information over a broad range of wavelengths all at once. "FTIR" stands for Fourier Transform Infrared, which is the mathematical process used to transform the collected data into an interpretable spectrum. FTIR spectroscopy is a method for figuring out infrared emission and absorption spectra. It is based on the measurement of the electromagnetic radiation absorption using mid-infrared wavelengths (4000-400 cm-1) [19]. The frequency of vibration between the bonds in the nanoparticle is represented by the absorption or emission peaks in the FTIR spectrum. The Fourier transform infrared spectrum is an excellent tool for qualitative analysis as the highest intensity indicates the nature of the components present in the sample.

3.3.**Ultraviolet Visible Spectroscopy**

Ultraviolet-visible (UV-Vis) spectroscopy measures how much distinct wavelengths of UV or visible light are absorbed by or transmitted through a material compared to a reference or blank sample. This technique can reveal the composition and concentration of components within the sample. The energy of light is inversely proportional to its wavelength, meaning shorter wavelengths carry more energy than longer ones. UV-Vis spectroscopy operates within the wavelength range of 190 to 1100 nm and is a straightforward and quick method for analyzing the spectra of small and nanoscale materials. Absorption occurs when electrons in a substance are promoted to higher energy states, which requires a specific amount of energy that corresponds to particular wavelengths of light. Because electrons in different bonding environments require varying amounts of energy for such transitions, this explains why different substances absorb light at different wavelengths.

Humans can only see a portion of the spectrum of visible light, which spans from approximately 380 nanometers (nm), or what we perceive as violet, to about 780 nm. UV radiation has wavelengths that are about 100 nanometers shorter than visible light. Since light may be classified according to its wavelength, UV-Vis spectroscopy can utilize this information to test or discriminate between different substances by pinpointing the particular wavelengths that correspond to their maximum absorption [20].

3.4.**Antibacterial Testing**

Antibacterial activity of Zein loaded Nanofibrous scaffold against E-coli was analyzed using common shake flask method. Escherichia coli (E.coli) bacteria was cultured in nutrient broth for 18 hrs at 98.6°F, and by the broth dilution method prepared solution was examined Bacterial cell concentration was adjusted to 1 × 10^9 CFU/mL and then serially diluted in 0.03 mol/L phosphate-buffered saline (PBS) to achieve concentrations ranging from 3 × 10^5 CFU/mL to 4 × 10^3 CFU/mL. Upon reaching the desired bacterial growth, 30 mg of the sample (zein-loaded nanofibrous scaffold) was added to a conical flask containing 65 mL of 0.3 mM PBS solution along with 5 mL of the prepared bacterial suspension. The mixture was then incubated on an electric shaker for 18 hours at 37°C.

Following incubation, 1 mL aliquots of the bacterial suspension at various concentrations were plated onto agar plates and further incubated for 24 hours at 37°C (98.6°F). After this period, the bacterial colonies on the agar plates were counted visually [21]. Antibacterial activity was assessed based on these results, and the reduction in bacterial colonies was calculated using the following equation:

$$R=\frac{(B-A)}{B}\times 100$$

Here;

R is the percentage of reduction in bacterial colonies, B is the number of bacterial colonies before treatment,

A is the number of bacterial colonies after treatment.

4. Results And Discussion

4.1. Morphological Study

The surface morphology of the electrospun nanofibers was examined using Scanning Electron Microscopy (SEM) at an accelerating voltage of 10 kV. The SEM images were captured at a magnification of 10,000×, with a scale bar representing 1 µm. This high magnification enabled detailed visualization of the fiber network and facilitated accurate measurement of fiber diameters for statistical analysis. The structural characteristics of clean nanofibers and nanofibers that included drugs were examined using SEM imaging. Figures 5.1 (a) and (b) show the SEM images of drug-loaded zein NFs made of pure zein polymer with curcumin and aloe vera, respectively. The zein nanofiber has an average diameter of 159 ± 20 nm. Aloe vera and curcumin were encapsulated into patches by increasing the diameter of drug-loaded nanofibers to 172±20 nm. The 35 nanofibers were randomly selected from the SEM micrographs and measured by the ImageJ software to determine the average fiber diameter. The smooth and bead-free structural qualities of the hybrid nanofiber patch were confirmed. This demonstrated that the drug and antimicrobial agent were evenly distributed over the nanofibrous patch. As shown in "Fig.1. (a1) and (b1)"

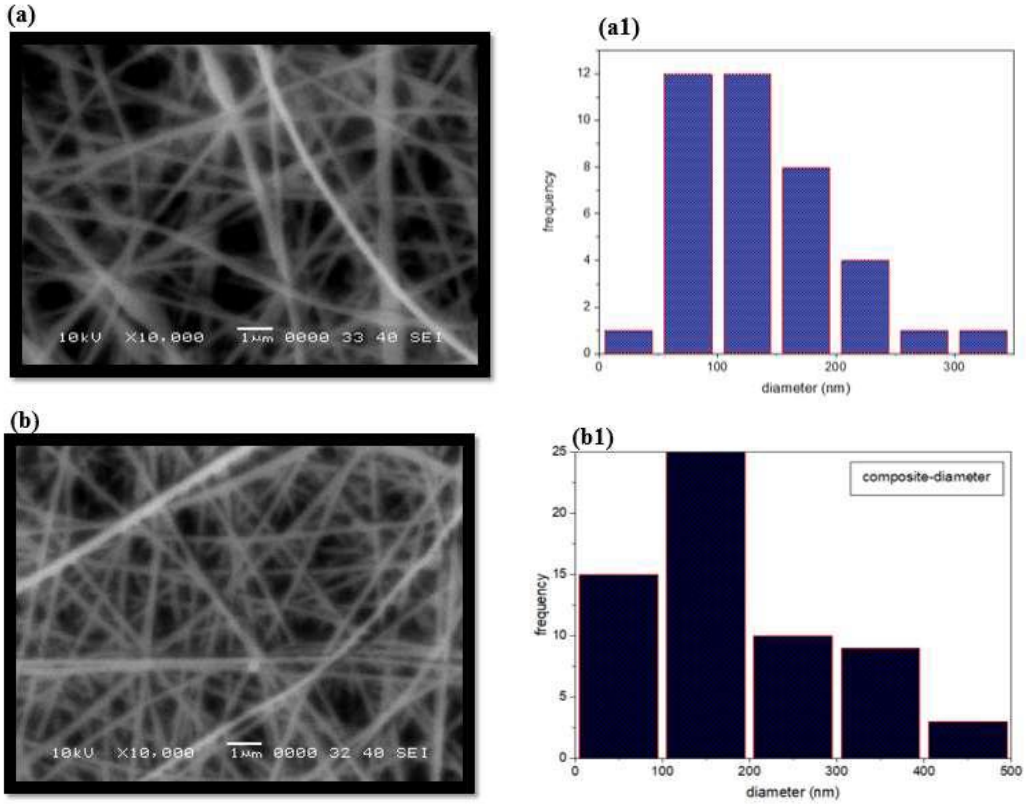

Fig. 1. SEM image of (a) pure zein nanofibers; (b) Aloe vera and curcumin nanofibers

4.2. FTIR Spectral Analysis

Curcumin-loaded nanofibers and plain nanofibers containing the antibacterial agent zein polymer aloe vera were analyzed using FTIR spectra, as shown in "Fig. 2 (a) and (b)". The changes in band positions with respect to neat zein nanofibers suggest interactions of C-O, C=C, and S=O, C=N groups of Cr and Av with Zein polymer.

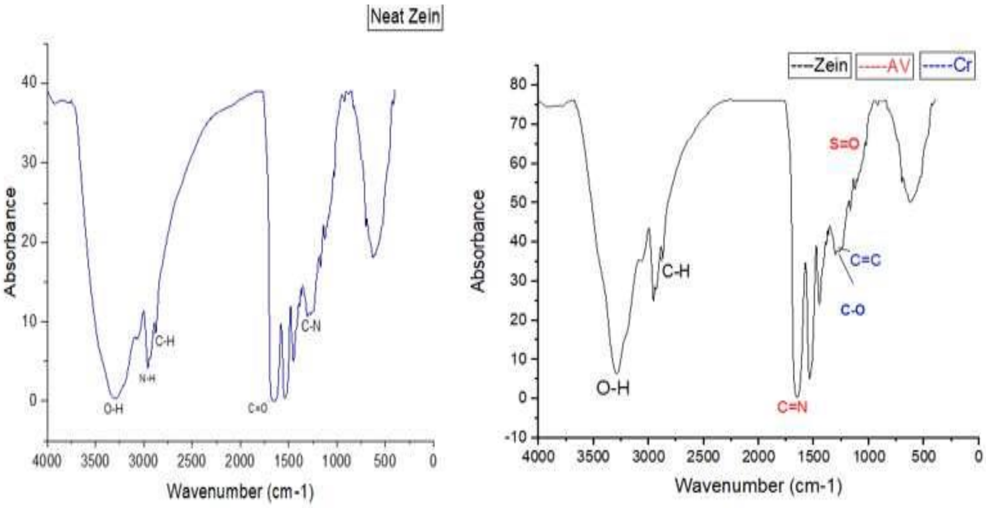

Fig. 2. (a) FTIR-pure Zein nanofibers (b) FTIR-Av/Cr loaded Zein nanofibers.

4.3.**Release Profile of Nanofibers**

To test the drug release profile, Phosphate-buffered saline (PBS) was prepared by adding 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of sodium phosphate, and 0.24 g of potassium dihydrogen phosphate to 1 liter of distilled water. The pH of the solution was adjusted to 7.2, and the mixture was stirred until all components were completely dissolved. The resulting solution was an isotonic PBS, commonly used in biological experiments and investigations [22]. A small patch of our nanofibrous sheet was immersed in PBS solution. A small patch of hybrid sheet was immersed in PBS solution, and then UV-vis spectrophotometry was carried out over multiple time spans; this was done to guarantee that the fresh samples showed cumulative release. The temporal release profile of aloe vera and curcumin from an electrospun zein NF patch is shown in "Fig. 3". It was observed that the drug was completely released within 60 minutes.

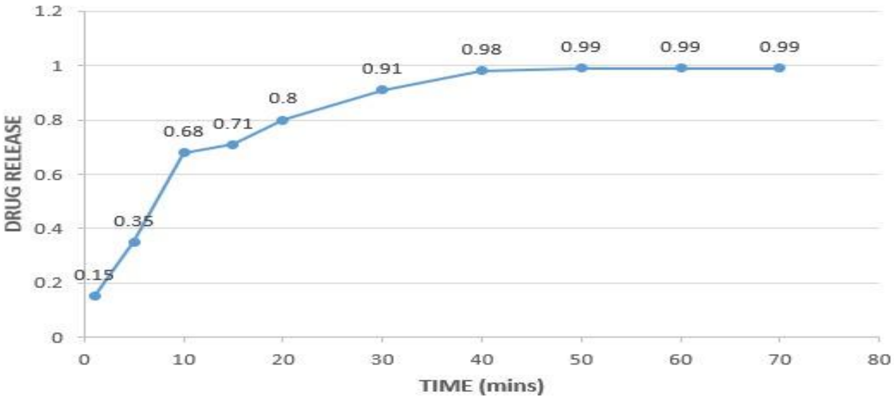

Fig. 3. UV-visible Spectra of Zein Encapsulating Aloe vera and Curcumin

4.4.**Antibacterial Activity**

Zein nanofibrous scaffolds were analyzed, with pure zein nanofibrous electrospun scaffolds serving as the control. Bacterial colonies were incubated in growth medium alongside drug-loaded zean nanofibrous patches, and their antibacterial properties were assessed using the shake flask method. The drug-loaded scaffolds exhibited significant antimicrobial activity. In contrast, the total density of microbial colonies on the pure zean scaffolds was high (as shown in Fig. 4), indicating no reduction in bacterial colonies. The highest reduction was observed in bacterial colonies treated with drug loaded zean nanofibrous scaffold. The results shows that Aloe vera and curcumin loaded zean nanofibers visible Reduction in bacterial colonies and calculated in "Fig. 4". These results suggest that any Aloe vera- curcumin loaded zean fibers were the cause of reduction of E. coli bacterial colonies.

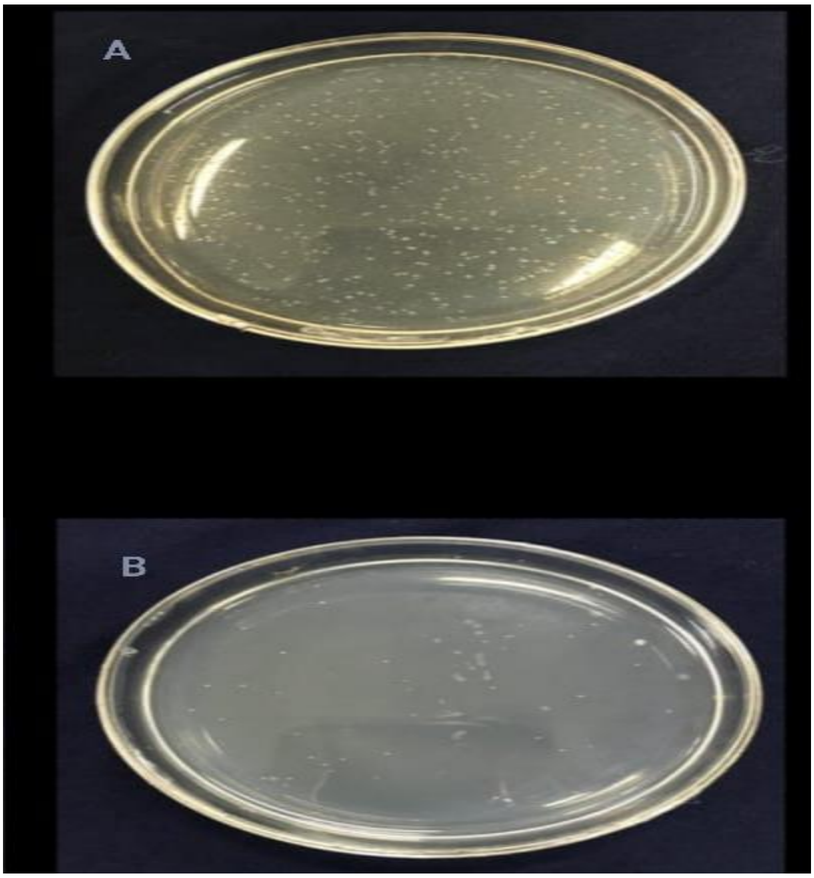

Fig. 4. Escherichia coli colonies (A) Untreated (B) treated with curcumin and aloe vera based nanofibers

5. Conclusion

We started with zein and DMF for polymeric base solution. Then we make it with Aloe vera in multiple concentrations to optimize its weightage in the nanofibrous sheet. Then we make it with curcumin with its multiple concentrations to optimize its weight. After preparing separate sheets of both drugs, then we make composite sheets of both of our drugs, i.e., aloe vera and curcumin. We saw zein nanofibers loaded with Aloe vera and curcumin were effectively created. The hybrid nanofibrous dressing's SEM micrographs showed a very smooth and bead-free morphology. The SEM images also showed homogeneous therapeutic agent encapsulation within and on top of nanofiber. Indicators of drug encapsulation can be seen in the slightly altered intensities and locations, as well as some novel absorption areas, in the FTIR spectra. Aloe vera and curcumin were released from zein, as shown by the release profile of hybrid nanofibers. In order to create a dressing for diabetic foot ulcers, the described Aloe vera and curcumin nanofibers loaded with zein are having promising delivery systems for the therapeutic agent's prolonged release and the antibacterial agent's burst release.

6. Future Work

This study focuses on developing a nanofibrous scaffold wound dressing for diabetic foot ulcers (DFU). The morphology, chemical composition, and drug release characteristics of the resulting nanocomposite were thoroughly examined. In the future, this research can be expanded to include in vivo testing, which may involve animal models such as rats, rodents, or rabbits. To ensure effective evaluation, animals with well-formed feet will be preferred.

For in vivo testing, a group of rats will be selected and administered alloxan with cold normal saline, followed by a 10% glucose solution. Alloxan induces liver damage, which impairs insulin production since the liver regulates glucose metabolism. This leads to insulin resistance and disrupted glucose homeostasis [23]. After preparing the subjects, a biopsy or fine cut will be made on the rat's foot, and the wound's depth and diameter will be measured.

The nanofibrous scaffold will be applied every 24 hours, with the wound size recorded daily. The healing process will be monitored over a period of at least 21 days. This approach could lead to the development of nanofiber membranes for medical DFU wound dressings on an industrial scale, improving DFU healing outcomes. After the trials, the effectiveness of this nanofibrous dressing will be compared with other synthesized and natural drug-loaded nanofibers to assess its overall efficiency.

Acknowledgement

The authors express their gratitude to Mehran University of Engineering and Technology Jamshoro, Pakistan's Textile Engineering and Biomedical Engineering departments for providing the lab space and facilities needed to carry out the required experimentation.

References

[1]. X. Zhang, D. Sun, G. C. Jiang. Comparative efficacy of nine different dressings in healing diabetic foot ulcer, A Bayesian network analysis, *J Diabetes*, Vol. 11, pp. 418-426, 2019.

[2]. Y. Liu, S. Zhou, Y. Gao, Y. Zhai. Electrospun nanofibers as a wound dressing for treating diabetic foot ulcer, *Asian Journal of Pharmaceutical Sciences*, Vol. 14, pp. 130-143, 2019.

[3]. C. W. Hicks, J. K. Canner, N. Mathioudakis, C. Lippincot, R. L. Sherman, C. J. Abularrage. Incidence and risk factors associated with ulcer recurrence among patients with diabetic foot ulcers treated in a multidisciplinary setting, *J Am Coll Surg*, Vol. 246, pp. 243-250, 2020.

[4]. E. J. Paul, B. Padmariya. A pragmatic review on the property, role and significance of polymers in treating diabetic foot ulcer, *Materials Today Proceedings*, Vol. 23, pp. 91-99, 2020.

[5]. K. Alexiadou, J. Doupis. Management of diabetic foot ulcers, *Diabetes Therapy*, Vol. 3, pp. 1-15, 2012.

[6]. S. Noor, M. Ahmed, J. Zubair. Diabetic foot ulcer—A review on pathophysiology, classification and microbial etiology, *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, Vol. 9, pp. 192-199, 2015.

[7]. I. George Broughton, J. E. Janis, C. E. Attinger. A brief history of wound care, *Plastic and Reconstructive Surgery*, Vol. 117, pp. 6-11, 2006.

[8]. L. Yazdanpanah, M. S. Literature review on the management of diabetic foot ulcer, *World J Diabetes*, Vol. 6, pp. 37-53, 2015.

[9]. L. I. Moura, A. M. Dias, E. Carvalho, H. C. De Sousa. Recent advances on the development of wound dressings for diabetic foot ulcer treatment, A review, *Acta Biomaterialia*, Vol. 9, pp. 7093-7114, 2013.

[10]. J. R. Hilton, D. T. Williams, B. Beuker, D. R. Miller, K. G. Harding. Wound dressings in diabetic foot disease, *Clin Infect Dis*, Vol. 2, pp. 100-103, 2004.

[11]. A. S. Fard, M. Esmaelzadeh, B. Larijani. Assessment and treatment of diabetic foot ulcer, *Int J Clin Pract*, Vol. 61, pp. 1931-1938, 2007.

[12]. Y. Sari. A comparative study of the effects of Nigella sativa oil gel and Aloe vera gel on wound healing in diabetic rats, *Journal of Evidence-Based Integrative Medicine*, Vol. 23, 2018.

[13]. S. A. Hashemi. The review on properties of Aloe vera in healing of cutaneous wounds, *BioMed Research International*, 2015.

[14]. T. A. Ngutyen Friedman, A. J. Curcumin: A novel treatment for skin-related disorders, *Journal of Drugs in Dermatology*, Vol. 12, pp. 1131-1137, 2013.

[15]. S. Bansal, S. Goel. Curcumin in wound healing: A concise review, *Wound Medicine*, Vol. 14, pp. 19-24, 2016.

[16]. A. B. Nair, S. Jacob. Curcumin solubility enhancement and its potential applications, *Pharmaceuticals*, Vol. 9, Issue 2, p. 47, 2016.

[17]. W. Lu. An update on molecular mechanisms of curcumin effect on diabetes, *Current Diabetes Reviews*, Vol. 10, pp. 14358, 2022.

[18]. S. Taheri, M. Ams, H. Bustamante, L. Vorreitter, M. Withford, S. Clark. A practical methodology to assess corrosion in concrete sewer pipes, *Construction and Building Materials*, Vol. 199, p. 6010, 2018.

[19]. T. Theophile. Infrared spectroscopy, *Materials Science, Engineering and Technology*, BoD, Books on Demand, 2012.

[20]. R. Asmatulu, W. S. Khan. Characterization of electrospun nanofibers, In *Synthesis and Applications of Electrospun Nanofibers*, Chapter 13, 2019.

[21]. H. Memon, A. A. Memon, A. Q. Ansari, A. A. Otho. Fabrication and characterization of Aloe Vera and tea tree oil loaded antimicrobial nanofiberous dressing, *Liaquat Med Res J*, Vol. 4, Issue 4, Available from: https://doi.org/10.38106/LMRJ.2022.4.4-04, 2023.

[22]. A. Q. Ansari, S. J. Ansari, M. Q. Khan, M. F. Khan, U. A. Qureshi, Z. Khatri, F. Ahmed, I. Kim. Electrospun Zein nanofibers as drug carriers for controlled delivery of Levodopa in Parkinson syndrome, *Materials Research Express*, Vol. 6, p. 075405, 2019.

[23]. P. Masiello, R. Lupi. Alloxan-induced diabetes and the role of liver in insulin resistance, *Diabetologia*, Vol. 46, Issue 4, pp. 502-551, 2003.