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Development of Biofunctionalized Composite Nanofibers by Electrospinning for Advanced Wound Healing Applications only

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Abstract

Wound healing is a complex biological process that is often impaired due to infection, chronic diseases, and aging, leading to prolonged non-healing wounds that pose a significant clinical challenge. Conventional wound dressings such as gauze and lint are limited in their ability to maintain a moist environment and prevent microbial infection, while modern and bioactive dressings offer improved therapeutic performance. In this study, a biofunctionalized composite nanofiber dressing was developed using the electrospinning technique for advanced wound healing applications. The proposed system integrates biopolymers such as chitosan, gelatin, and curcumin, selected for their complementary properties including biocompatibility, structural support, antimicrobial activity, anti-inflammatory effects, and antioxidant behavior. The electrospun nanofibers closely mimic the extracellular matrix (ECM), thereby promoting enhanced cell adhesion, proliferation, and tissue regeneration. Process optimization using the OFAT method enabled the fabrication of uniform, bead-free nanofibers under optimized parameters of voltage, flow rate, and spinneret distance. Morphological analysis using SEM confirmed the formation of continuous nanofibrous structures, while FTIR spectroscopy validated the successful incorporation of bioactive compounds without undesirable chemical interactions. The results demonstrate that the developed composite nanofiber dressing provides a multifunctional platform for efficient wound healing by addressing infection control, inflammation reduction, and tissue regeneration, making it a promising candidate for next-generation biomedical wound care applications.

Keywords: Electrospinning; Nanofibers; Wound Healing; Chitosan; Gelatin; Curcumin; Bioactive Dressing.

How to Cite This Article

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Introduction

Wound healing is a complex, dynamic and biological cascade that is necessary to restore the integrity of the skin and its protective capability [1, 2]. It is done in a co-ordinated cascade of haemostasis, inflammation, proliferation, and tissue remodelling [3]. Nevertheless, it is a natural process of repair which can be impaired due to such factors as infection, aging, or systemic diseases, such as diabetes, and cause the occurrence of chronic wounds. They are non-healing wounds that provide a significant clinical and economic burden to the healthcare systems every time around the world, undermining patient quality of life and necessitating extensive and intensive care [4, 5].

Nanotechnology has risen to the challenge and has become an area of breakthrough in contemporary wound care [1, 6]. The electrospinning method is one of the techniques that have become prominent due to its capability to produce polymer nanofibers that highly resemble the structure of the native extracellular matrix (ECM) [5, 7]. These nanofibers have high surface-area/ volume ratio and tenable porosity forming the best scaffold that increase cell adhesion, proliferation and differentiation, and therefore creates the best environment in which a tissue can regenerate [5].

The current study is aimed at developing a new composite nanofiber dressing through a strategic mixing of bioactive materials. Chitosan, a natural polysaccharide, is chosen because of its bio-compatibility, bio-degradability, as well as its anti-microbial and haemostatic effects [8, 9]; gelatine, the derivative of collagen, is selected due to its structural scaffold, which allows the adhesion and growth of cells [10, 11]; curcumin is a strong anti-inflammatory, antioxidant, and antimicrobial natural compound which is selected due to the presence of a structural scaffold [3].

The overall objective of the present research is hence to integrate and define a new, bio functionalized composite nanofiber dressing, which will take advantage of the interaction of these constituent materials to produce a multifunctional wound healing dressing. In order to form the scientific precedent of this work, the next section will examine the history of wound dressing and the therapeutic logic of the biomaterials chosen.

Literature Review

This review will give a critical evaluation of the scientific environment that guides the formulation of the proposed composite nanofiber dressing. It sets the scientific standard about such materials by following the development of wound care materials using traditional ones and the development to modern and bioactive ones. Moreover, it reviews the peculiarities of the chosen biopolymers, including chitosan, gelatin, curcumin, and rutin, to explain the reasons why these biopolymers should be incorporated into one electrospun matrix, thus, determining the research gap in this work.

The Evolution of Wound Dressings

The moist healing environment theory, which defined the moist environment as a faster tissue repairing condition, is the basis of the paradigm of modern wound care [15]. This has been used to create modern wound dressing that is capable of optimizing the healing environment [4, 16]. There are widely used wound dressings, which are divided into the traditional, modern, and bioactive classes; each of them is one of the large steps made in the functionality.

Common dressings, e.g., gauze and lint, are not expensive and are commonly found. Their shortcomings are, however, very noticeable; they have a tendency to fail to seal the extrudate of the wound, and stick to the wound bed upon removal leading to the secondary tissue destruction, and provide poor protection against bacteria invasion [17, 18]. Modern dressings on the other hand are designed to overcome these disadvantages. The type of material in this category is specifically wound-specific. Wound monitoring with film dressings is clear, whereas foam dressings have the best exudate absorption [19, 20]. Hydrocolloids create a gel when they interact with the wound fluid and induce healing, and hydrogels keep the dry wounds moist [21, 22]. Based on seaweed, the dressing is known as alginate, which is very absorbent and suited to wounds that are heavily exuding [23].

Bioactive dressings represent the state of the art since present. The following sophisticated materials are actively involved in the healing process [24]. Made of biopolymers such as chitosan and gelatin and usually loaded with therapeutic factors, they stimulate cell migration, fight infection, and regulate inflammation, making them an essential method of complex and chronic wounds management [25]. Modern and bioactive dressings are similar in the sense that modern dressing is better in terms of moisture control nature, whilst bioactive dressing is most advanced in terms of the functionality in which it promotes tissue regeneration.

Rationale for Selected Biomaterials in Electrospinning

The constituent materials of a bioactive dressing are fundamental determinants of the performance of the dressing. The choice of chitosan, gelatin, curcumin, and rutin within this composite system is ingrained in the well-reported and complementary therapeutic qualities. Chitosan A natural polysaccharide that is produced by the conversion of chitin, chitosan is characterized by its bio-compatibility, bio-degradability, and non-toxicity [8, 9]. Its main benefits in wound healing are due to its strong and broad-spectrum anti-microbial action which helps to prevent wound infection and hemostatic effects which facilitates blood clotting.

Gelatin This is a denatured collagen, which is the main protein of the ECM, a perfect biomaterial in tissue engineering [11]. It comprises bioactive sequences which aid in the important cellular processes, such as adhesion, migration and proliferation [10, 26]. The presence of gelatin in the nanofibers enables the dressing to replicate the natural tissue environment which gives a favorable environment to enable the regeneration of the tissue.

Curcumin Curcumin is a poly-phenolic molecule that is tripartite in properties i.e. anti-inflammatory, antioxidant, and antimicrobial [3]. It assists to regulate the inflammatory reaction, demilitarize destructive reactive oxygen species (ROS), and prevent bacterial development. One of the most significant issues of curcumin is its low aqueous solubility but when embedded in nanofibers, the curcumin is overcome by its solubility, making it possible to deliver it locally and continuously to its wound site [27, 28].

Rutin A is a naturally-occurring flavonoid that supplements the effect of curcumin with its own powerful antioxidant and anti-inflammatory effects [13]. It reduces the oxidative stress, which is a major cause of pathology in chronic wound, by scavenging free radicals. It has been demonstrated to be incorporated into chitosan-based nanofibers to produce effective antibacterial dressings [14]. Although the literature validates the personal advantages of these four components, the originality of this study is uniting them into one system of electrospun nanofibrils of composite materials. This approach will design a dressing that combats the complex issue of wound healing infection, inflammation, and tissue damage via a multi-pronged approach. The sections below define the exact procedure of preparing and describing this new system.

Materials and Methods

This section details the precise experimental protocols employed in this study, from material selection and solution preparation to process optimization and analytical characterization. The methods described herein were designed to ensure the study's scientific rigor and reproducibility.

Materials

The following materials were used for the synthesis of the nanofibers:

- Gelatin
- Chitosan
- Curcumin
- Rutin
- Formic acid (solvent)

Preparation of Polymer Solutions and Concentration Rationale

The preparation of a homogeneous polymer solution is a critical precursor to successful electrospinning. Polymer solutions were prepared based on target weight-by-volume (w/v) concentrations, and the final weight-by-weight (w/w) concentrations were calculated based on the measured masses of the solutes and the solvent (formic acid, density = 1.22 g/mL). This approach accounts for the density of the solvent, providing a precise measure of solute concentration in the final solution. The final calculated w/w concentrations are summarized for each formulation in the table below.

Table 1. *Formulation and Final Solute Concentration (%)*

Formulation	Final Solute Concentration (%)
Gelatin	19.74%
Gelatin + Curcumin	14.69%
Gelatin + Chitosan	14.69%
Gelatin + Rutin	14.98%

Electrospinning Process Optimization

An initial stage of pilot experimentation was carried out to determine that nanofiber on appropriate levels of electrospinning. The One-Factor-at-a-Time (OFAT) method was utilized to make a systematic variation of a single parameter (flow rate, voltage, or distance) when the rest of the parameters were held constant.

Early experimentation demonstrated that pure chitosan, rutin or curcumin could not be spinned in pure conditions alone under the conditions of the experiment because of a lack of chain entanglement. Gelatin needed a minimum target concentration of 30 percent w/v and voltage threshold of at least 18 kV to form fibres. Its concentrates with the other components at lower concentrations (approximately 14.69% 14.98 w/w) but over a smaller working range. Under this optimization stage, a number of technical issues were experienced as indicated below.

Table 2. *Electrospinning Process Challenges and Limitations*

Problem Type	**Description**
Low Spinnability	Especially for pure biopolymers (chitosan, rutin, curcumin) due to poor chain entanglement.
Viscosity Too Low or Too High	Either fails to form fibers (too low) or causes jet issues/beading (too high).
Narrow Operating Windows	Especially for blends; small deviations in concentration lead to failed fiber formation.
Jet Instability at High Flow	Seen consistently at flow rates ≥0.8–0.9 ml/hr.
Voltage Threshold Dependency	Fiber formation only above a certain threshold (≥18 kV).
Short Spinneret-to-Collector Distance	Insufficient flight time below 11 cm led to no fiber formation.

Based on the systematic OFAT optimization, the following final process parameters were established for the successful synthesis of uniform nanofibers:

- Flow Rate: 0.5 ml/hr
- Voltage: 25 kV
- Spinneret-to-Collector Distance: 15 cm

Characterization of Nanofibers

To assess the morphological and chemical characteristic of the synthesized nanofibers, the following analytical techniques were used.

- Scanning Electron Microscopy (SEM): The morphology of the surface was examined using Scanning Electron Microscopy and the fiber diameter of the synthesized nanofibers was determined. The procedure enables the production of high-resolution images to determine the establishment of continuous and bead-free fibrous structure.
- Fourier Transform Infrared Spectroscopy (FTIR): FTIR was used to determine the chemical functional groups of the nanofibers. This analysis proves the chemical structure as well as confirms the adequate incorporation of all the ingredients into the polymer structure without undesired chemical reactions.

Results

This part is the major research findings of the experiment and is devoted to morphological and chemical characteristics of the successfully created nanofibers. The findings have empirical evidence of the optimization of the electrospinning process and the creation of a biofunctionalised composite material.

Morphological Analysis of Nanofibers (SEM)

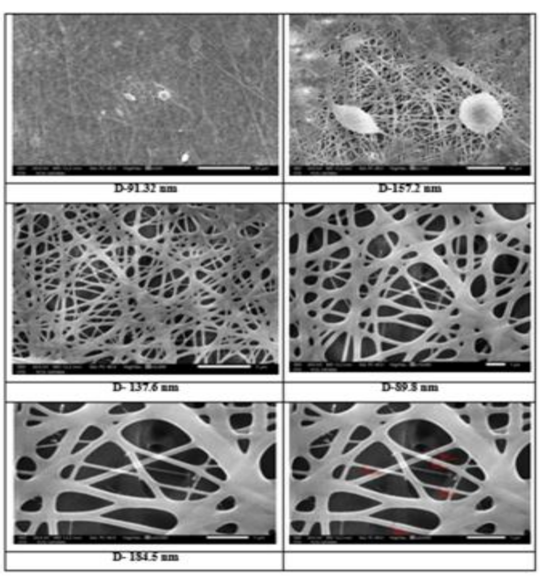

Fig. 1*. SEM micrograph of electrospun Gelatin nanofibers at the optimized parameters*

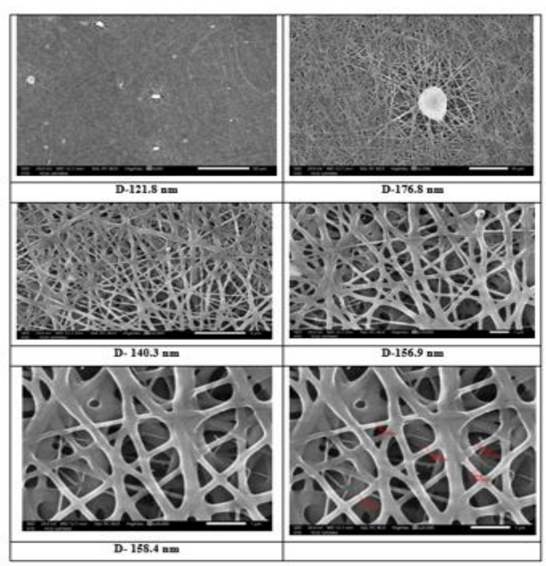

Fig. 2*: SEM micrograph of electrospun Gelatin-Curcumin composite nanofibers*

To determine the fiber size distribution, the diameters of the individual fibers were determined based on the SEM images and the results are shown in Table 1. The mean diameter of the pure gelatin nanofibers was 132.1 + 38.2 nm with a gelatin-curcumin composite fibers having a mean diameter of 150.8 + 21.1 nm. This affirms that the fabricated structures are very much more than the nanometer scale which is essential to give the high surface-area-to-volume ratio that is beneficial to the wound healing applications.

Table 3. *Measured Fiber Diameters from SEM Analysis*

Sample	**Mean Fiber Diameter (nm)**
Gelatin Nanofibers	132.1 ± 38.2
Gelatin-Curcumin Nanofibers	150.8 ± 21.1

Chemical Composition Analysis (FTIR)

FTIR spectroscopy was performed to confirm the chemical composition of the electrospun mats and to verify the successful incorporation of curcumin into the gelatin matrix. The FTIR spectra for the pure gelatin and gelatin-curcumin samples are shown in Fig. 3 and Fig. 4, respectively

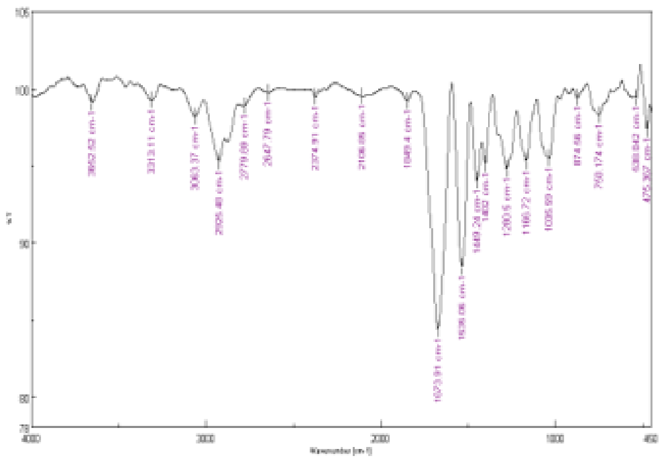

Fig. 3. *FTIR spectrum of pure Gelatin nanofibers.*

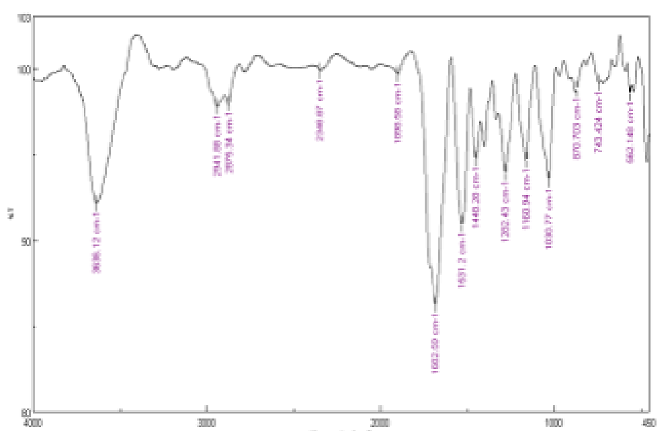

Fig. 4. *FTIR spectrum of Gelatin-Curcumin composite nanofibers*

The spectrum for pure gelatin nanofibers exhibited characteristic absorption peaks that confirm the polymer's structural integrity was preserved during the electrospinning process. Key peaks, as detailed in Table 2, include a broad band around 3313 cm^{-1} corresponding to N–H and O–H stretching, the amide I band (C=O stretching) at approximately 1674 cm^{-1}, and the amide II band (N–H bending) at approximately 1535 cm^{-1}. The presence of these signature peaks validates that the gelatin maintained its fundamental chemical structure.

Table 4. *Key FTIR Absorption Peaks and Their Assignments for Gelatin Nanofibers*

Wavenumber (cm^{-1})	**Interpretation**
3313.11	N–H and O–H stretching
2925.48	C–H stretching
1673.91	Amide I (C=O stretching)
1535.06	Amide II (N–H bending)
1280.50	Amide III

In the spectrum for the gelatin-curcumin composite nanofibers, the characteristic peaks of gelatin were again present. Alongside these, subtle shifts and additional minor peaks associated with curcumin were observed, confirming its presence within the fiber matrix. The analysis indicates that curcumin was physically incorporated into the gelatin polymer without forming new covalent chemical bonds.

Conclusion

This study was able to achieve this main goal in that it formulated and described a biofunctionalized nanofiber composite that could be used in wound healing using electrospinning method. The experiment revealed the optimization of the electrospinning conditions, and pure gelatin and composite gelatin-curcumin nanofibers with no beads were produced. The completed process specifications (25 kV voltage, 0.5 ml/hr flow rate, 15 cm distance) were strong enough in the production of quality fibrous mats.

The most important results of the characterization studies give good evidences of the applicability of the material to the purpose of its use. SEM analysis proved the presence of the desired nanofibrous morphology that is necessary to mimic the extracellular matrix and allow cell interaction. Moreover, the FTIR analysis confirmed the chemical composition of the gelatin and the physical-chemical integrity of the gelatin and physically introduced curcumin on the nanofibers without any negative chemical interactions. The implications of this work on the broader scope of the field of advanced wound care are important.

This research paper forms a critical methodological basis towards the eventual objective of the research program to come up with a synergistic four-component system that would integrate chitosan, gelatin, curcumin and rutin. This kind of composite would provide a multi-pronged treatment regimen, which would help in terms of infection, inflammation and tissue regeneration. The results are a positive step in the right direction, to fill the gap between the ability to make biomaterials to regenerative medicine on a laboratory scale and the creation of the next generation of biomaterials that are clinically relevant.

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