Synthesis and Characterization of Polyvinylidene Fluoride/Curcumin/*Chlorella vulgaris* Composite Nanofibers

Balanehru Subramanian[1*], *Alvin* Thomas[1], *Ratnakar* Arumugam[1*], *Renuga* Mohan[2], *Canivizhi* Vijayamannane[3], *Sabarirajan* Jayaraja[1], and *Lizha* Mary[1]

[1]School of Biomedical Sciences, Sri Balaji Vidyapeeth (Deemed to Be University), Puducherry 607402, India.

[2]Allied Health Sciences, SRM Medical College Hospital and Research Centre (Affiliated to The Tamilnadu Dr. M. G. R. Medical Univeristy, Chennai), Tiruchirapalli, India.

[3]School of Chemistry and Forensic Science, Division of Natural Sciences, University of Kent, Canterbury CT2 7NZ, United Kingdom.

Abstract. Polyvinylidene fluoride (PVDF) is a promising polymer for such applications but requires enhancement to address limitations in antimicrobial activity and biocompatibility. This study investigates the incorporation of curcumin and *Chlorella vulgaris* into PVDF nanofibers to improve these properties. A total of five composite nanofiber samples were prepared by electrospinning PVDF, followed by post-treatment soaking in ethanol solutions containing varying concentrations of curcumin and *Chlorella vulgaris*. The resulting nanocomposites were characterized using X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). The results revealed significant improvements in antimicrobial activity, with the composite containing 15% curcumin and 3% *Chlorella vulgaris* demonstrating the most effective performance. These findings suggest that the incorporation of curcumin and *Chlorella vulgaris* into PVDF nanofibers provides a promising solution for enhancing antimicrobial properties and biocompatibility, offering potential for advanced wound healing applications.

1. Introduction

The increasing demand for advanced biomaterials in biomedical applications, particularly in wound healing and tissue regeneration, has spurred the exploration of new composite materials with enhanced mechanical, antimicrobial, and biocompatible properties [1, 2]. Polyvinylidene fluoride (PVDF) is one such polymer that has garnered significant attention due to its exceptional chemical stability, mechanical strength, and biocompatibility [3, 4]. However, despite these advantageous properties, PVDF in its pure form exhibits limited antimicrobial activity, which is crucial for applications in wound healing and infection control. To overcome these limitations, there is a growing interest in developing PVDF-based composites that incorporate bioactive agents capable of promoting healing and resisting microbial infections. In this context, the addition of curcumin and *Chlorella vulgaris* into PVDF matrices offers a promising solution due to their inherent bioactive properties. The management of chronic wounds, such as those resulting from burns, diabetes, and surgical incisions, tumour removal remains a significant challenge. These wounds are often prone to bacterial infections, delayed healing, and tissue degeneration. Traditional wound dressings, while effective to some extent, fail to address the multifaceted needs of such wounds, especially the prevention of bacterial growth and the promotion of tissue regeneration [2]. Thus, there is a critical need for advanced wound dressings that not only provide a physical barrier but also possess inherent antimicrobial properties and promote healing by supporting tissue regeneration. Compared to biodegradable polymers such as PCL or PLA, PVDF offers superior mechanical stability, resistance to hydrolytic degradation, and sustained structural integrity in moist wound environments. Moreover, the inherent piezoelectric behaviour of PVDF has been reported to promote cellular responses relevant to tissue regeneration, making it an attractive candidate for multifunctional wound dressing systems

PVDF has emerged as a promising candidate due to its high mechanical strength, ease of processing, and biocompatibility. However, its lack of antimicrobial properties limits its effectiveness in controlling infections in wound healing applications. The incorporation of natural antimicrobial agents such as curcumin and *Chlorella vulgaris* into PVDF composites can address this limitation. Curcumin, a polyphenolic compound derived from the rhizome of Curcuma longa, has been widely studied for its anti-inflammatory, antioxidant, and antimicrobial properties [5-7]. *Chlorella vulgaris*, a green microalga, has also demonstrated antimicrobial activity and has the potential to enhance the overall biocompatibility of the composite material [10, 11].

To create a highly mesoporous effective antimicrobial wound dressing, various material synthesis and processing techniques can be employed. Conventional methods, such as solution casting, membrane development processes (e.g., foam or sponge making), and fiber-based materials, have been extensively used in the preparation of biomaterials [1, 5]. Solution casting involves dissolving a polymer in a solvent and allowing it

* Corresponding author: subramanianb@sbvu.ac.in

to form a film upon solvent evaporation. While this process is relatively simple, it often results in dense films with low surface area and limited porosity, which restricts their ability to promote cellular growth and enhance antimicrobial properties. Another common method is membrane development through the creation of foams or sponges. These processes involve the formation of a porous structure, either by the use of foaming agents or by inducing phase separation during the polymerization process. The resulting foams and sponges offer higher porosity compared to films, facilitating improved cell infiltration and fluid absorption, which are essential for wound healing applications. However, the mechanical strength and the uniformity of the pores can be challenging to control, potentially affecting the material's overall performance. While solution casting and foam-making processes have their merits, they fall short in creating highly porous structures with a large surface area, which is crucial for biomedical applications. Electrospinning, a method that produces nanofibers through the application of a high electric field, offers significant advantages over traditional techniques in this regard. Electrospinning creates continuous nanofibers with diameters ranging from tens to hundreds of nanometers, resulting in a highly mesoporous structure [4-6]. These nanofibers have an extremely high surface area-to-volume ratio compared to films, foams, and sponges, which enhances the material's potential for cellular interactions, drug loading, and antimicrobial properties. The electrospinning technique allows for the precise control of fiber morphology and porosity, which are crucial for applications such as wound healing and scaffolding in tissue engineering. Additionally, electrospun mats can be designed to mimic the extracellular matrix (ECM), providing a scaffold for cell adhesion, migration, and proliferation [6, 13]. This level of control over nanofiber morphology and surface properties makes electrospun nanofibers particularly attractive for biomedical applications, including wound dressings and scaffolds for organ repair.

Incorporating bioactive agents like curcumin and *Chlorella vulgaris* into the PVDF nanofiber matrix can significantly enhance the material's functionality [11-13]. The controlled release of bioactive agents such as curcumin and *Chlorella vulgaris* from the electrospun nanofibers can provide sustained therapeutic effects, reducing the frequency of dressing changes and enhancing patient comfort [12]. Curcumin has long been recognized for its wide range of therapeutic effects, particularly its antimicrobial, anti-inflammatory, and antioxidant properties. In wound healing, curcumin has been shown to promote tissue regeneration by reducing inflammation and oxidative stress, which are common in chronic wounds. Moreover, curcumin's antimicrobial properties help prevent bacterial infections, a critical aspect of wound healing. By incorporating curcumin into the PVDF matrix, these bioactive properties can be imparted to the composite material, offering dual benefits: mechanical support and antimicrobial resistance [14]. *Chlorella vulgaris*, on the other hand, is a green microalga that has been studied for its beneficial properties, including antimicrobial activity, wound healing promotion, and immune system modulation. *Chlorella vulgaris* is rich in essential amino acids, vitamins, and minerals, making it a valuable component in promoting cellular regeneration and tissue repair [15]. When incorporated into the PVDF matrix, *Chlorella vulgaris* can enhance the biocompatibility of the composite and further boost its antimicrobial efficacy. The combination of curcumin and *Chlorella vulgaris* in a PVDF nanofiber matrix creates a multifunctional material that addresses both infection control and tissue regeneration, making it highly suitable for use in advanced biomedical applications, including bone and wound healing.

2. Materials and Methods

Materials

Polyvinylidene Fluoride (PVDF) powder (average Mw ~180,000 by GPC), (Sigma-Aldrich, India), Curcumin ((≥95% purity), (Merck, India) , *Chlorella vulgaris* extract (in house prepared & characterized), analytical-grade Dimethylformamide (DMF), Ethanol (Merck, India) was procured and used as such without further purification and processing.

Methods

2.2.1 Synthesis of PVDF nanofibers and incorporation of curcumin and *Chlorella vulgaris*

The PVDF/curcumin/*Chlorella vulgaris* composite nanofibers were fabricated using an electrospinning, a widely used technique for producing nanofibers with high surface-area-to-volume ratios. The setup housed inside a sterile chamber that is dedicately used only for the preparation of biomaterials free from metal oxides. Initially, PVDF was dissolved at 15% (w/v) in DMF, while curcumin was added at 5–15% (w/w relative to PVDF content). The solution was stirred for 24 hours to ensure complete dissolution of the polymer. To prepare the composite, varying concentrations of curcumin and *Chlorella vulgaris* were added to the PVDF solution. A total of five composite nanofiber mats were prepared, namely: Sample 1 (PVDF with 5% curcumin) (C1-P5-C0), Sample 2 (PVDF with 10% curcumin and 1% *Chlorella vulgaris*) (C2-P10-C1), Sample 3 (PVDF with 15% curcumin and 3% *Chlorella vulgaris*) (C3-P15-C3), Sample 4 (PVDF with 5% curcumin and 5% *Chlorella vulgaris*) (C4-P5-C5), and Sample 5 (PVDF with 10% curcumin and 10% *Chlorella vulgaris*) (C5-P10-C10).

The electrospinning process was carried out using a high-voltage electrospinning setup. The electrospinning parameters included an applied voltage of 18 kV, a needle gauge of 22, a flow rate of 0.3 mL/h, and a tip-to-collector distance of 15 cm. The electrospun fibers were collected on a rotating drum with aluminium foil collector, and the spinning time was optimized to ensure uniform fiber formation.

2.2.2 Post-treatment soaking process

Following the electrospinning process, the nanofiber mats were subjected to a post-treatment soaking procedure to further incorporate curcumin and *Chlorella vulgaris* into the nanofiber matrix. The mats were immersed in ethanol solutions containing curcumin and *Chlorella vulgaris* at varying concentrations for 24 hours. Ethanol (90%) was used as the solvent to facilitate the dissolution of curcumin and extract *Chlorella vulgaris*. The soaking process allowed the bioactive agents to diffuse into the nanofiber structure, enhancing their integration into the composite material. After the soaking process, the nanofiber mats were air-dried for 24 hours to remove any residual ethanol and excess curcumin or *Chlorella vulgaris*. The post-treatment soaking process ensures additional loading of the bioactive compounds and also to compensate the loss of the bioactive compounds that occurred during the spinning process, if any. The nanofiber mats were dried at 37° C, inside a sterile incubator.

Characterization of the materials

The structural, morphological, and functional properties of the PVDF/curcumin/*Chlorella vulgaris* composite nanofibers were thoroughly evaluated using a range of characterization techniques. To investigate the crystalline structure of the nanocomposites, X-ray diffraction (XRD) was performed using a Rigaku X-ray diffractometer (Japan). XRD patterns were recorded over a 2θ range of 10°–60°, and the crystallinity of the nanocomposites was evaluated to confirm the incorporation of curcumin and *Chlorella vulgaris* into the PVDF matrix. The FTIR spectra of the nanofibers were obtained using a Fourier-transform infrared (FTIR) spectrometer (Thermo Scientific, USA) in the range of 4000–500 cm-1. FTIR analysis helped identify the chemical functional groups present in the composite, as well as the interaction between PVDF, curcumin, and *Chlorella vulgaris*. Thermogravimetric analysis (TGA) was used to evaluate the thermal stability of the composites. A TGA analyzer (TA Instruments, USA) was employed, and the samples were heated from 30°C to 600°C at a rate of 10°C/min under nitrogen atmosphere. TGA provided insights into the degradation behavior and stability of the PVDF-based nanocomposites, especially with respect to the incorporation of bioactive agents. Scanning electron microscopy (SEM) was used to examine the morphology of the electrospun nanofibers. SEM images were captured using a FEI Quanta 200 microscope (USA) to observe the fiber diameter, uniformity, and the overall network structure of the nanofibers. SEM analysis also provided insights into the porosity and surface texture of the composite materials. The antimicrobial properties of the nanocomposite nanofibers were evaluated using doubling time assay against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, as well as against fungal strains (*Candida albicans*) [16-18]. An increase in microbial doubling time relative to the control was considered indicative of effective antimicrobial activity. The doubling time was spectroscopically investigated to determine the antimicrobial efficacy of the nanofibers, providing information on their potential for infection control in wound healing applications.

Results and discussions

3.1 Results

3.1.1 X-Ray Diffraction (XRD) and Fourier Transform Infra-Red spectroscopy studies (FTIR)

The phase and structure of the prepared electrospun nanofiber mat were investigated by XRD and FTIR. The XRD peak patterns obtained for the prepared materials are presented in figure 1. From figure 1, the XRD patterns of pure PVDF and the composite nanofibers revealed semi-crystalline with dominant amorphous characteristics. As, broad diffraction halos are characteristic of electrospun polymer nanofibers. Also, the incorporation of curcumin and *Chlorella vulgaris* did not significantly affect the crystalline phase of PVDF. The diffraction peaks of PVDF at 2θ = 17.5°, 18.5°, and 20.7° remained intact in all composite samples, indicating that the crystalline structure of PVDF was preserved after the incorporation of bioactive agents [5]. However, slight shifts in the diffraction peaks were observed, suggesting the formation of interactions between PVDF, curcumin, and *Chlorella vulgaris*, which is consistent with the successful integration of the bioactive agents into the polymer matrix.

3.1.2 Thermal Stability of the Nanocomposites

The thermal stability of the prepared materials were was analyzed using thermogravimetric analysis (TGA). The TGA curves obtained are presented in the figure 3. The TGA curves for the pure PVDF and composite samples showed similar thermal degradation behaviour, with the onset of degradation occurring at approximately 350°C. The mass loss observed above 350°C is attributed to the decomposition of PVDF. The composite samples displayed a slight reduction in thermal stability compared to pure PVDF, which can be attributed to the presence of curcumin and *Chlorella vulgaris*.

This reduction in thermal stability is expected, as bioactive agents generally have lower thermal degradation points compared to the polymer matrix. However, the composite nanofibers retained a significant proportion of their mass at higher temperatures, indicating that the incorporation of curcumin and *Chlorella vulgaris* did not drastically reduce the thermal stability of the PVDF matrix.

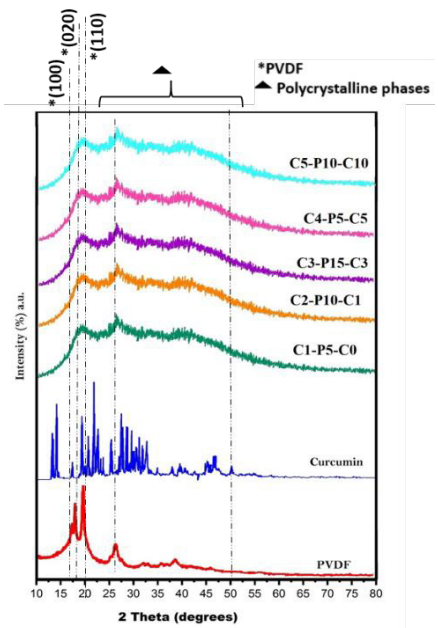

Fig. 1. XRD patterns obtained for the PVDF, curcumin and the prepared materials.

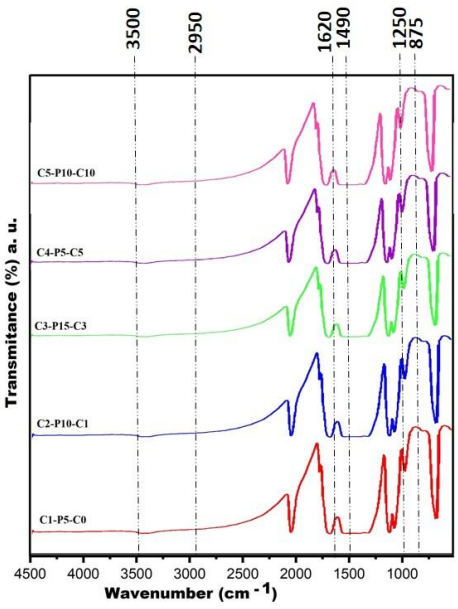

Fig. 2. FTIR spectra of the prepared materials.

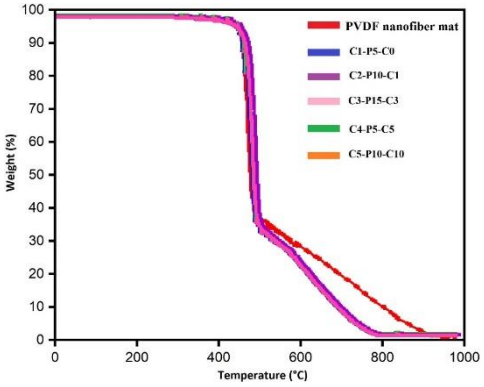

Fig. 3. TGA curves obtained for the PVDF electrospun nanofiber and the other prepared materials.

3.1.3 Scanning Electron Microscopy (SEM)

The morphology of the electrospun C3-P15-C3 nanofibers was evaluated using scanning electron microscopy (SEM). The SEM images revealed that the electrospun PVDF fibers were uniform in diameter and formed a continuous, non-woven mat structure.

The average fiber diameter of pure PVDF was approximately 200–300 nm. In the composite samples, the incorporation of curcumin and *Chlorella vulgaris* did not significantly alter the fiber morphology; however, some variations in fiber diameter were observed depending on the bioactive agent concentration. The nanofibers in the composite samples exhibited diameters ranging from 200 to 400 nm. The surface of the fibers appeared smooth and homogeneous, with no significant beading or defects, indicating successful electrospinning without significant disruption from the incorporated bioactive agents.

The presence of curcumin and *Chlorella vulgaris* does not appear to affect the overall fiber morphology in a disruptive manner. The smooth surface of the fibers suggests that the bioactive agents were well-dispersed within the PVDF matrix and did not cause significant changes to the nanofiber formation process. This is particularly important because any aggregation or uneven distribution of the bioactive agents could have compromised the performance of the composite, particularly its ability to promote cell adhesion or release bioactive compounds efficiently.

The homogeneous distribution of the bioactive agents within the nanofibers likely ensures consistent antimicrobial and regenerative effects, which are desirable for wound healing applications.

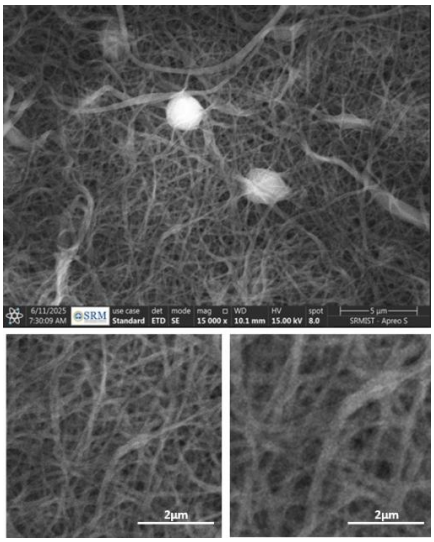

Fig. 4. SEM images obtained for C3-P15-C3 nanofibers.

3.1.4 Antimicrobial Properties of the Nanocomposites

The antimicrobial properties of all the prepared materials were assessed using the doubling time assay against common bacterial strains (*Staphylococcus aureus (MTCC 3160)*, *Escherichia coli (MTCC 1687)*, and fungal strains (*Candida albicans (MTCC 227)*. The results obtained are presented in figure 5.

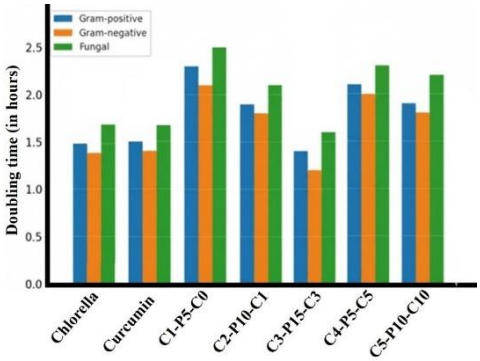

Fig. 5. Doubling time analysis of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* in the presence of PVDF and PVDF/curcumin/*Chlorella vulgaris* composite nanofibers. An increase in doubling time indicates inhibition of microbial growth.

The results indicated that the composite nanofibers exhibited significantly improved antimicrobial activity compared to pure PVDF. Among all the prepared samples, C3–P15–C3 exhibited the most pronounced antimicrobial activity, as evidenced by a significant increase in microbial doubling time compared to the control and other composite formulations. Similarly, *Candida albicans* exhibited a marked increase in doubling time, indicating effective inhibition of fungal growth. The enhanced antimicrobial activity is attributed to the combined effects of curcumin, known for its antimicrobial properties, and *Chlorella vulgaris*, which contributes to antimicrobial activity through its bioactive compounds, such as peptides, lipids, and polysaccharides [8].

The incorporation of both bioactive agents significantly boosted the antimicrobial properties of the PVDF matrix, making the composite nanofibers particularly effective for infection control in wound healing applications.

3.2 Discussion

The results of this study demonstrate that the incorporation of curcumin and *Chlorella vulgaris* into PVDF nanofibers significantly enhances their antimicrobial properties, without adversely affecting the structural integrity of the nanofibers. The XRD and FTIR analyses confirmed the successful incorporation of both bioactive agents into the PVDF matrix, with interactions between the polymer and the bioactive agents that are likely responsible for the enhanced antimicrobial activity. The electrospinning technique proved to be an effective method for producing highly porous nanofiber mats with a high surface area, which is critical for promoting cellular interactions in biomedical applications. The smooth and uniform fiber morphology observed in the SEM images is ideal for tissue engineering and wound healing applications, where cell adhesion and proliferation are essential for tissue regeneration. The antimicrobial activity observed in the composite nanofibers suggests that they are effective in preventing bacterial and fungal infections, a major concern in wound healing. Among all the prepared composite materials, Sample C3–P15–C3 demonstrated pronounced antimicrobial activity property compared to the other materials. The observed extension of microbial doubling time in the presence of the composite nanofibers confirms their effective growth-inhibitory behavior. The increased doubling time reflects delayed cell division resulting from sustained exposure to bioactive compounds released from the nanofiber matrix.This enhanced activity can be attributed to the higher curcumin loading, which provided a stronger and more sustained antimicrobial and antioxidant effect, and to the efficient release of bioactive compounds from *Chlorella vulgaris* embedded within the highly mesoporous structure of the nanofiber mat. The mesoporosity facilitated better diffusion of the active agents, ensuring effective interaction with microbial cells and promoting overall biological activity [20, 21]. The absence of similar property in the rest of the prepared materials can be attributed to the reduced curcumin content and the absence of a well-developed mesoporous framework. Insufficient curcumin loading limit the availability of active molecules at the surface, while less porous structures restrict the diffusion and release of Chlorella-derived biomolecules.

4. Conclusion

Thus the present research work demonstrates the fabrication of PVDF/curcumin/*Chlorella vulgaris* composite nanofibers using electrospinning, followed by a post-treatment soaking process to incorporate curcumin and *Chlorella vulgaris* into the nanofiber matrix. The resulting composite nanofibers exhibited enhanced antimicrobial activity, with the composite containing 15% curcumin and 3% *Chlorella vulgaris* showing the best performance against both bacterial and fungal strains. The incorporation of curcumin and *Chlorella vulgaris* did not compromise the structural integrity of the PVDF nanofibers, as evidenced by the XRD, FTIR, and SEM analyses, which confirmed successful integration of the bioactive agents without altering the fiber morphology. The PVDF/curcumin/*Chlorella vulgaris* composites demonstrated significant antimicrobial properties, making them highly suitable for applications in wound healing and infection control. The high surface area and porosity of the electrospun nanofiber mats facilitate cell adhesion, migration, and proliferation, which are essential for tissue regeneration. Additionally, the bioactive agents contribute to the therapeutic benefits of the composites, including anti-inflammatory and antioxidant effects, which are beneficial for wound healing.

These findings suggest that PVDF/curcumin/*Chlorella vulgaris* composite nanofibers have great potential for advanced biomedical applications, such as wound healing, bone repair, and scaffolds for organ regeneration. Future studies could explore the optimization of the formulation for sustained release of bioactive agents, as well as further in vivo studies to assess the full therapeutic potential of these composites in clinical settings.

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