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Research Article

Development of Poly-vinyl alcohol (PVA) and *Moringa oleifera*-based nanofibre patches for wound healing using an indigenous electrospinning setup

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Abstract

Wound healing is a crucial process that underlines the body's ability to recover and restore damaged tissues. Conventional cotton-based dressings act as a physical barrier between the environment and open wound and are not actively involved in wound healing. This type of dressing can lead to maceration of scab, and infection and can cause pain upon removal. Functionalized nanofibre patches that can accelerate wound healing are being researched worldwide to overcome these challenges. Electrospinning has emerged as the most promising technique for engineering nanofibres for diverse applications. In this study, the wound-healing potential of PVA nanofibres functionalized with different concentrations (0.1%, 0.2%, 0.3%, 0.5%, and 1 wt%) of Moringa oleifera (MO) extracts are explored. This study provides insights into the influence of different parameters such as polymeric solution concentration, needle size, tip-to-collector distance, the high voltage applied, and types of collectors on nanofibre production. These parameters are optimized to produce nanofibre patches using a self-assembled indigenous electrospinning setup. The produced nanofibres were characterized and the average diameter of unloaded and loaded PVA nanofibres was found to be ~125 nm and ~450 nm respectively.

Keywords: Electrospinning, PVA, *Moringa oleifera*, wound healing

I. Introduction

Conventional wound dressings merely serve as a physical barrier between the environment and the open wound, lacking the ability to accelerate the wound healing process. Furthermore, such dressings can inadvertently contribute to issues like scab maceration, infection, and discomfort during removal. Researchers are exploring the potential of functionalized nanofibre patches to address these challenges and expedite wound healing. These patches boast a high surface area to volume ratio, offering promising prospects for enhancing the wound healing process [1].

MO often referred to as the 'miracle tree,' is a member of the Moringaceae family. Predominantly found in tropical and sub-tropical regions worldwide [2], it is renowned for its diverse medicinal properties. Owing to its abundant nutritional content, MO is extensively incorporated into food products and various

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industries as a dietary supplement. The leaves of MO are particularly rich in bioactive compounds, including phenols, flavonoids, proteins, and polysaccharides, as well as essential vitamins and minerals. These compounds show antagonistic effects against tumours, reactive oxygen species (ROS), oxidative stress, bacteria and microbes, and diabetes mellitus. These properties are essential for wound healing [3]. PVA is a commonly used polymer that is water-soluble, biodegradable, and biocompatible [4]. It is often used to make functionalized nanofibres that have potential biomedical applications. Mostly, the production of such nanoscaled fibres is conducted using a method called electrospinning. Electrospinning is a versatile technique; it employs an electrically charged jet of polymer to fabricate fibres whose diameter is on the nanometer scale. The major components of an electrospinning setup consist of a syringe pump, a collector, and a high-voltage supply [1]. The syringe pump pushes the polymer solution loaded in the syringe and ensures a steady flow of the solution through a blunt needle which acts as a spinneret. The collector plate is either grounded or oppositely charged to the spinneret, and the high-voltage supply is connected to the needle. The polymeric solution forms a 'Taylor cone' at the needle tip. Electric charges overcome the solution's surface tension, causing it to stretch and form nanofibres. These fibres move towards the collector propelled by the potential difference. This study evaluates the performance of an indigenous electrospinning setup developed in the laboratory. The parameters of the electrospinning process were optimized to produce fibres on a nanoscale.

MO was extracted from MO leaves using the soxhlet extraction method. Total phenolic content (TPC) and total flavonoid content (TFC) tests were employed to characterize the functional properties of the extracts. The MO-loaded PVA nanofibres were produced and characterized using SEM, FTIR, and XRD analysis.

II. Literature review

Numerous studies have proven the potential of nanofibres in biomedical applications [1]. Brahatheeswaran et al., developed hybrid nanofibre mats of zein and curcumin which were ideal for soft tissue regeneration and drug delivery [5]. Similarly, poly (ε-caprolactone) and keratin-based nanofibres were synthesized by Edwards et al., for regenerative medicine and tissue engineering [6].

MO extracts, particularly their leaf extracts are well-known for their wound healing capabilities. Their properties have been used to aid the healing of not only acute wounds but also diabetic wounds. They have been used to functionalize PVA hydrogels [7] and/or poly (acrylonitrile) nanofibres [8]. Furthermore, studies were conducted by Chin et al., on hyperglycaemic animal models, wherein MO extracts were used to promote diabetic wound healing [9].

PVA has been extensively utilized for many years as a substrate for electrospinning pharmaceuticals. Zein and tragacanth gum, for instance, have been used by Ghalei et al., and Thamer et al., respectively, to produce functionalized PVA nanofibre patches [10, 11]. Ibrahim et al. studied the inhibitory effects on butyrylcholinesterase (BuChe), monoamine oxidase A (MAO A), and monoamine oxidase B (MAO B) enzymes using electrospun MO-loaded PVA nanofibres with different concentrations (0%, 0.1%, 0.2%, and 0.4%) of leaf extracts of MO. This study showed that the inhibitory effect of the nanofibres increases with a higher concentration of MO extracts [12]. Therefore, in this study, MO and PVA-based patches were produced using an indigenous electrospinning setup while optimizing the quality of nanofibres produced.

III. Methodology

a. Preparation of alcoholic extracts

Fresh leaves of MO were surface sterilized with 0.2% mercuric chloride and heat dried at 37^0C in hot air for 24 hours. The leaves were dried till a constant weight was obtained and then ground into a fine powder and stored in an airtight container. Soxhlet extraction of MO was performed using ethanol and methanol as solvents in a 1:10 ratio (powder: solvent) at 60^0C for eight hours. This step was followed by rotary evaporation to obtain a concentrated alcoholic crude extract [13].

b. Solution preparation

i. PVA solution preparation: PVA procured from Sigma-Aldrich (USA) (Product no.: P8136, molecular weight = 30,000 – 70,000, 87-90% hydrolysis) was utilized to prepare the polymer solution at a concentration of 20% (20 g in 100 ml) in HPLC water. The PVA solution underwent magnetic stirring at 60^0C for two hours [14]. Subsequently, it was placed in a self-assembled vacuum desiccator to eliminate any foam generated during stirring. This step was pivotal to ensure proper loading of the polymer solution into the syringe.

ii. MO-PVA solution preparation: The ethanolic crude extracts were used to make the loaded polymer solutions as they had higher TPC and TFC values (ref section IV). One g of MO extract was added into 100 ml of 20% PVA to make 1% MO + PVA. This solution was further stirred for ten mins and vacuum desiccated to remove any foam. The one wt.% MO-PVA solution was then diluted to make solutions of 0.1, 0.2, 0.3, 0.5, and 1 wt.% concentration [12].

c. TPC and TFC of the extracts

TPC of the solutions was measured to quantify the total amount of phenolic compounds present in the extracts. It was done using the Folin-Ciocalteu method [15]. For calculating the total amount of flavonoids present in the ethanolic extracts, TFC was measured using aluminium chloride [16].

d. Electrospinning

Table 1 illustrates the different concentrations of PVA which were electrospun under various parameters to optimize the pure PVA nanofibre production before any functionalized patches were prepared. A self-assembled indigenous electrospinning setup that consisted of a syringe pump (SPLab01) connected to a high-voltage supply and a control system was used (Fig 1). The flow rate on the syringe pump varied from 0.1ml/hr to 1 ml/h. The high voltage supply was varied in the range of 9 to 16 kV. The various needle sizes were tested viz., 20 G, 21 G, 23 G, and 25 G. A 6 ml syringe (internal diameter = 12 mm) was loaded with the polymer solution and connected to a connector tube with the needle. Electrospinning was also performed at various gap distances, i.e., the distance between the needle tip and the collector. Gap distances varied between 5 and 9 cm. Besides these parameters, multiple collectors were also tested. Aluminum foil, OHP sheet, non-woven fibrous sheet, cling wrap, and parchment paper were tested for collectors.

Table 1: Parameters of the Electrospinning process which were optimized to produce stable nanofibres

PVA sol. conc.	Needle size (G)	Gap Distance (cm)	Voltage (kV)	Flow Rate (ml/h)	Observation of nanofibres
5%	20 to 25	5 to 9	9 to 16	0.1 to 1	No
8%	20 to 25	5 to 9	9 to 16	0.1 to 1	No
10%	20 to 25	5 to 9	9 to 16	0.1 to 1	YES
20%	20 to 25	5 to 9	9 to 16	0.1 to 1	YES
30%	20 to 25	5 to 9	9 to 16	0.1 to 1	No

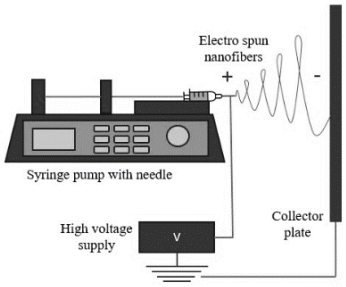


Fig 1. Schematic of indigenous electrospinning setup

e. Characterization of the nanofibre patches

Log-normal distribution of nanofibres was calculated from the SEM images taken by a scanning Electron Microscope (SEM-EDS) (Hitachi SU 3500). FTIR and XRD graphs were obtained using ATR (Diamond) Fourier Transform Infrared (FTIR) Spectrometer (Shimadzu, IRAffinity-1S) and Powder X-ray Diffractometer (PXRD) (Bruker, D8 ADVANCE ECO), respectively.

f. Statistical analysis

Statistical analysis was performed using a t-test to assess the differences in values between the TPC results of ethanolic and methanolic extracts. Specifically, the t-test was employed to compare the amount of phenolic compounds extracted by each of the two solvents and to establish which provided better extraction. This analysis allowed for the evaluation of the statistical significance of any observed differences, which had a significance level set at $p < 0.05$. The statistical software utilized for data analysis was GraphPad Prism 9.2.0.332. Additionally, the results are presented as mean difference with 95% confidence intervals (CI) where appropriate or as mean ± standard deviation (SD)

IV. Results and analysis

a. Characterization of alcoholic extracts

1) **Total Phenolic Content and Total Flavonoid Content:** Fig 2a illustrates the TPC values for ethanolic and methanolic extracts of MO. The TPC values for ethanolic extracts (28.103 ± 0.52 mg GAE/ g) were notably higher than those of methanolic extracts (22.148 ± 0.54 mg GAE/ g) ($p = 0.004$). This discrepancy could be attributed to the enhanced solubility of MO bioactive components in ethanol [17]. In comparison with the findings of Fachriyah et al., our study yielded lower TPC values for ethanolic extracts. This disparity may stem from differences in the sourced samples [18]. TFC, on the other hand, was performed only for the ethanolic extracts since they showed a higher TPC result. This was performed in triplicates (Fig 2b).

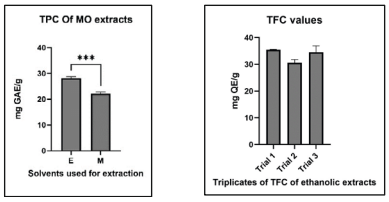


Fig 2. a) TPC measurement of methanolic and ethanolic MO extracts. b) TFC measurement of ethanolic extracts.

b. Physical properties of the solutions

i. **Viscosity and conductivity:** Viscosity and conductivity are two essential solution parameters that directly affect the electrospinning process. The viscosity of the solution determines if the solution will undergo electrospinning or electrospraying. Similarly, electrical conductivity directly influences the voltage the solution will be subjected to produce nanofibres [19]. However, there is no available literature on the range of viscosity and conductivity at which a solution may undergo electrospinning and produce nanofibres of the desired diameter range. The optimal viscosity and conductivity depend upon the electrospinning conditions and thus, needs to be optimized. The optimal viscosity and electrical conductivity range for this setup were measured to be between 16 cP to 20 cP and 1.4 Mho/cm to 1.6 Mho/cm, respectively.

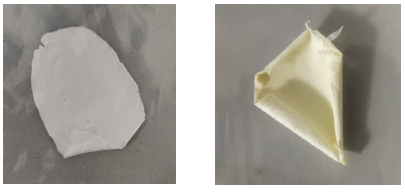
c. Optimization of nanofibre production

Since the electrospinning setup was self-developed, multiple parameters had to be

optimized to produce the best quality nanofibres. Initial trials established that the ideal flow rate range for this setup was between 0.1 ml/h and 1 ml/h. It was observed that for every 1 cm of gap distance, 2 kV voltage was necessary for stable fibre formation. When spun with 20 G, 21G, and 23 G needles, nanofibre formation was often interrupted by microdroplets. Thus, further trials were conducted only with a 25 G needle which had an internal and external diameter of 0.26 and 0.515 mm; respectively, as it showed to help in the formation of stable nanofibres.

As depicted in Table 1, trials of optimization were conducted using pure PVA solutions of varying concentrations. Among them, 20% PVA solution was observed to provide the best nanofibre mats when electrospun at 0.1 ml/h and 9 kV with a needle of size 25 G and gap distance of 7.5 cm. Thereby, this solution was chosen as the base solution for the addition of MO extracts and the same conditions were maintained to produce nanofibres of the same quality.

Among all the collectors tested, parchment paper provided the best nanofibre mats after peeling. Figs 3a and 3b illustrate the best 20% PVA and 1% MO + 20% PVA patch obtained.



(a) (b)

Fig 3. Optimized nanofibre mats of (a) 20% PVA solution and (b) 1% MO + 20% PVA solution, respectively

d. Characterization of nanofibres

i. **SEM results:** SEM analysis of the nanofibre mats showed that the diameters of the nanofibres followed log-normal distribution. The diameters of pure PVA nanofibres varied from 61 nm to 301 nm with the average diameter being ~145 nm. In the case of MO-loaded patches, the nanofibre diameters varied from 259 to 806 nm and the average diameter was ~490 nm.

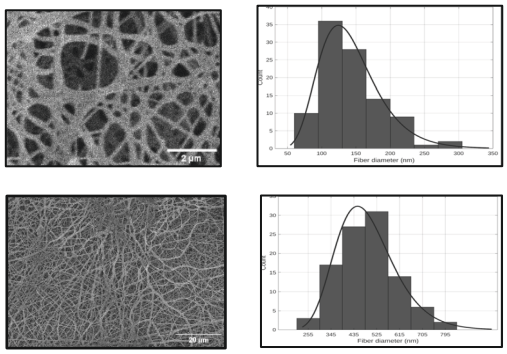
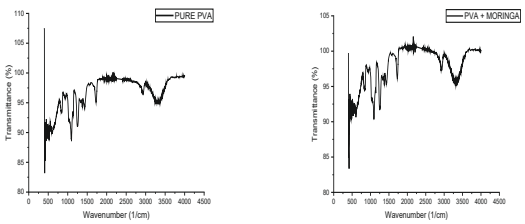


Fig 4. (a) SEM image of pure PVA patch, (b) The log-normal distribution curve of pure PVA patch, (c) SEM image of MO + PVA patch, (d) The log-normal distribution curve of MO + PVA patch

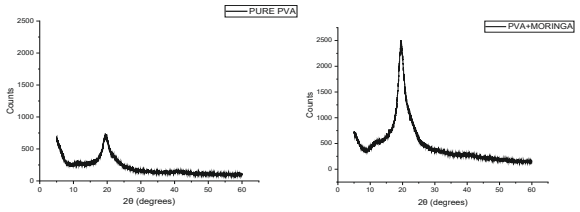
2) **FT-IR and XRD:** FT-IR and XRD measurements validated the presence of MO extracts within the patches. FT-IR spectrum was scanned within the wavelength range of 400 - 4000 $cm^{-1.}$ The spectra show an O-H stretching between 2750 – 3000 cm^{-1}. In Fig 5, both the spectra have similar peaks and there is no new peak seen in the MO + PVA spectra. However, the spectra of MO + PVA is observed to be slightly broader than the spectra of pure PVA [12].

Fig 6 illustrates the diffraction spectra of nanofibres of pure PVA and PVA+MO. Both the spectra show characteristic peaks at 20^0. The XRD graph of the MO + PVA patch showcased a sharper peak than the pure PVA patch implying that amorphousness increased due to the presence of MO [12].



(a) (b)

Fig 5. (a) FT-IR spectra of pure PVA patch, (b) FT-IR spectra of MO + PVA patch



(a) (b)

Fig 6. (a) XRD graphs of pure PVA patch, (b) XRD graph of MO + PVA patch

V. Conclusion

In this study, electrospinning was performed by experimenting with various parameters such as polymer solution concentration, needle size, tip-to-collector distance, the high voltage applied, and types of collectors to optimize the production of nanofibres for reproducibility. The TPC and TFC results of the nanofibre patches indicated the presence of phytochemicals in MO extracts, which indirectly indicated the functionality of the nanofibres and thereby, their potential to accelerate wound healing. The structural elucidation of the pure PVA and functionalized nanofibres was observed in the graphs obtained from FT-IR and XRD. These results further validated the presence of MO extracts in the functionalized patches. Log-normal distribution of the SEM results concluded that the nanofibres produced by the self-assembled indigenous electrospinning setup were in the nanoscale range, validating the functionality of the indigenous setup. In the future, assays such as scratch assay, disk diffusion, etc., need to be performed to establish the wound healing properties of these MO + PVA patches.

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