RESEARCH ARTICLE

Design and Assessment of *Boswellia* Nanofibers for Diabetes Wound Healing

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ABSTRACT

Diabetic wounds present a major healthcare challenge because they frequently result in serious side effects and chronic, non-healing ulcers. The application of nanotechnology in the creation of sophisticated wound dressings has demonstrated encouraging outcomes in accelerating the healing of wounds. Particularly, nanofiber-based dressings have drawn interest because of their large surface area, porosity, and capacity to replicate the extracellular matrix, which creates the perfect surroundings for attachment of cells, growth, and differentiation. This review emphasizes the various materials and methods utilized in the preparation of nanofiber dressings, with a particular focus on their application in the treatment of diabetic wounds. Herbs and plant extracts are among the synthetic and natural polymers that have been added to nanofibers to improve their medicinal qualities. These materials provide mechanical support and possess bioactive components that promote wound healing, such as anti-inflammatory, antimicrobial, and antioxidant properties. Boswellia resin from *Boswellia serrata*, PVP, and PCL was obtained, and nanofibers were electrospinning to form them. The formulated nanofibers were further evaluated for entrapment efficiency, swelling index and moisture content. The *in-vitro* study showed formulation NF3 has the highest drug release. scanning electron microscopy (SEM) studies revealed a uniform surface of nanofiber mat.

Keywords: Electrospinning, Wound healing, *In-vitro* permeation, *In-vivo* wound study.

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INTRODUCTION

There are 422 million people worldwide who have diabetes; most of them live in lower- and middle-class countries. Every year, it is solely accountable for 1.6 million deaths. Diabetic wounds occur in approximately 20% of the diabetic population. Diabetes mellitus frequently results in diabetic wounds, also referred to as diabetic foot ulcers, which are identified by sluggish or impaired wound healing.[1] Patients with diabetes often get these wounds on their feet, which can be caused by a number of things, such as immunological dysfunction, poor circulation, and neuropathy. Diabetic patients may experience a decrease in awareness of wounds or areas of pressure that may result in wounds due to neuropathy or damage to the nerves, which can cause loss of feeling in the feet. Peripheral arterial disease, or poor circulation, reduces blood flow to the extremities, which hinders the delivery of the nutrients and oxygen needed for wound healing. It can also deteriorate the immune system, causing it to be more difficult for the body to fight off diseases, which can postpone healing even longer.[2]

Diabetes-related wounds are difficult to treat and frequently call for a multidisciplinary strategy that includes infection control, offloading, and debridement—the elimination of dead or infected tissue and a reduction in pressure on the wound. In order to encourage healing and stop infection, modern wound dressings are also utilized, such as dressings based on nanofibers. Wound dressings based on nanofibers have become a viable treatment option for a variety of wounds involving diabetic wounds.[3] Such dressings imitate the framework of the extracellular matrix (ECM) framework present in human tissues by using nanoscale fibers, which usually have sizes varying from tens to hundreds of nm. A significant benefit of nanofiber wound dressings is their capacity to produce a moist wound environment, which is necessary to encourage the migration, proliferation, and differentiation of cells and to facilitate the interchange of nutrients and oxygen.[4,5] Nanofibers' large surface area-to-volume ratio also permits the regulated release of bioactive substances, which can improve healing even more. Examples of these substances include growth

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factors, antimicrobials, and anti-inflammatory medications. *Boswellia serrata*, also known as Indian frankincense, was used in the formulation of nanofibers. We gathered the polymers PCL and PVA. Boswellia extract helped improve wound healing in diabetic rats by reducing inflammation and promoting the formation of newer blood vessels in the wounded region, causing angiogenesis.[6] This accelerates wound closure in diabetic patients.

MATERIALS AND METHOD

Boswellia was purchased from IndiaMart. Polycaprolactone (PCL) was obtained from Thermo Fisher Scientific, India. Polyvinyl alcohol (PVA) is obtained from Chemanalyst. Ethanol and dimethyl sulfoxide (DMSO) were obtained from the Central Drug Store.

Extraction

Boswellia resin is ground into a fine powder using a mortar and pestle. It was then extracted using ethanol as solvent.[7] The extract was then concentrated and stored in a dark, airtight container at a cool temperature to maintain its stability and potency.

Electrospinning of Nanofiber

All the ingredients were weighed accurately (Table 1). The *Boswellia* extract was dissolved in ethanol. PVA and PCL polymers were each dissolved in DMSO and mixed uniformly for two hours using a magnetic stirrer. After mixing the two polymeric solutions, the *Boswellia* extract solution is gradually added while spinning at 500 rpm on a magnetic stirrer. The syringe was then filled with the formulation.[7] At a voltage of 20 KV, the nanofiber membrane was electrospun onto aluminum foil while keeping a distance of 18 cm between the needle and the collector.[8] After that, the nanofibers were carefully removed from the foil and their effectiveness in treating diabetic wounds was assessed.

Evaluation Studies

FTIR evaluation

FTIR spectra were recorded using an infrared spectrophotometer (Perkin Elmer Model No. 234). In the 400 to 4000 cm^{-1} range, the spectrum was recorded at a resolution of 2 cm^{-1}. Functional groups were determined, and various peaks were observed.

Entrapment efficiency

Electrospun nanofibers are expected to have a higher drug entrapment efficiency (%EE) because of their huge surface area.

Table 1: Formulation design for fabrication of nanofibers

Ingredients	*NF1*	*NF2*	*NF3*	*NF4*
Boswellia extract (gm)	0.2	0.2	0.2	0.2
Polycaprolactone (PCL) (gm)	0.25	0.75	1	1.5
Polyvinyl alcohol (PVA) (gm)	1	1.5	1.75	2
DMSO (mL)	2	2	2	2
Ethanol (mL)	7	7	7	7

EE provides a description of how effectively the medication is prepared for integration onto the carrier system.[9] Weighing and letting the drug-loaded nanofiber patch dissolve in a 7.4 phosphate buffer. The solution was tested in triplicate for the amount of entrapped drug utilizing a UV spectro-photometer at 412 nm. The calculation was done using the below formula for the percentage of drug EE:

$$EE\% = \frac{Entrapped\ drug}{Total\ amount\ of\ drud} \times 100$$

Percentage of moisture content

Each resulting nanofiber have been weighed and held for a full day at ambient temperature in a desiccator containing fused calcium chloride. After a full day, the films were weighed again.

$$\%Moisture\ content = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Swelling index

After being completely dry and pre-weighed, the *Boswellia* nanofibers were allowed to equilibrate in 250 mL phosphate buffer (pH 7.4) at 37°C. An analytical balance is used to measure the water absorption of the films after a 24-hour period.[10] The following equation is used to calculate the swelling ratio (Q) of the films:

$$Q = \frac{W_s - W_d}{W_d}$$

Where Ws is weight of the swelling film at various time intervals, whereas W_d is the weight of the dry film.

Scanning electron microscopy evaluation

The morphology of prepared and plain nanofiber patches was analyzed using IIT Kanpur's TESCAN (Model MIRA-3 LMH). The formulation was vacuum coated with gold sputter layer and mounted on metal ends utilizing double-sided adhesive tape prior to review.[11] Utilizing image analysis software, the diameters and distributions of the electrospun fibers were investigated from the scanning electron microscopy (SEM) images.

In-vitro drug release

A 20 mL receptor compartment capacity Franz-diffusion cell was used for *in-vitro* skin penetration experiments placed in between the diffusion cell's donor and receptor compartments is a semi-permeable membrane. After applying the prepared patches to the skin, paraffin film was covered. A pH 7.4 phosphate buffer with 20% alcohol was added to the diffusion cell's receptor compartment. All of the components were secured onto a magnetic stirrer. A magnetic bead was used to continuously stir the solution in the receptor compartment at a speed of 50 revolutions per minute while keeping the temperature constant at 37 ± 0.5°C.[12] At predefined intervals (0.5, 1, 2, 3, 4, 5 hours), samples were taken out and reconstituted with the dissolving medium before being examined for drug content utilizing a UV visible spectrophotometer at 412 nm. The graph was plotted.

Model-dependent kinetic modelling

Many mathematical functions describe the dissolution profile that form the foundation of model-dependent approaches. Following the selection of an appropriate function, the dissolution profiles are assessed using the obtained model parameters.[13]

In-vivo wound-healing

Wistar rats were selected for *in-vivo* wound-healing evaluation. The rat was anesthetized using ketamine hydrochloride, and a standardized wound was created on the skin (e.g., excisional, incisional, or burn wound) using a surgical procedure.[13] One rat was left untreated to observe natural healing, one was subjected to blank nanofiber with only polymers while another was subjected to nanofiber along with drug and polymers. The wound-healing process was monitored over time. Observations were carried for approximately 10 days.[14,15] The wound size was measured on the 1st, 5th, and 10th days, and the average reduction in wounds was obtained.

$$\%\ wound\ reduction = \frac{W_o - W_t}{W_t} \times 100$$

RESULTS AND DISCUSSION

The electrospinning nanofibers were evaluated and observed (Table 2). The nanofibers showed entrapment efficiency ranged from 94.15 to 97.06%. Nanofiber, NF3 showed maximum entrapment efficiency, which is crucial for the release of drug in a controlled manner for sustained therapeutic effect and minimizing side effects. The percentage moisture content ranged between 1.71 to 2.09%. The swelling index of prepared nanofiber was observed. The value ranges between 13.05 to 18.21%, where nanofiber, NF3 showed maximum swelling index reflected by drug permeation in *in-vitro* release. A high swelling index indicates that the nanofiber has a high capacity for absorbing fluids, which can be beneficial in wound dressings, where the nanofiber needs to absorb exudates from the wound.

FTIR Studies

The PVA spectrum shows the bands at 2793 cm^{-1} (CH2 stretching), 2806 cm^{-1} (CH3 stretching), 1791 cm^{-1} (–C=O stretching) and 901 cm^{-1} (CH2 rocking). Another related band at 1104 cm^{-1} is the stretching of CO and bending at 3510 cm^{-1} is assigned to the stretching of the hydroxyl group. In the fourier-transform infrared spectroscopy (FTIR) spectrum of PCL showed C=O Stretching and C-H Stretching at 1896 cm^{-1} and 2891 to 2901, respectively. Peak around 1186 to 1278 cm^{-1} was observed due to the stretching vibration of the C-O-C ether linkage. C-C Stretching is observed at 1288 cm^{-1}. FTIR of *Boswellia* showed a broad peak around 3309 to 3600 cm^{-1} because of the stretching vibrations of hydroxyl groups (O-H) in alcohols and phenols. C-H stretching and C=O stretching were observed at 2811 and 1700 to 1735 cm^{-1}, respectively. FTIR of prepared nanofiber showed no new peak formation. There was a slight shift in peaks, which confirmed the interaction between polymers during nanofiber formation.

Table 2: Evaluation parameters of formulated nanofibers

Formulation code	*Entrapment efficiency (%)*	*Moisture content (%)*	*Swelling index (%)*
NF1	94.15	1.71	13.05
NF2	95.83	1.77	15.67
NF3	97.06	1.84	18.21
NF4	96.04	2.09	16.17

Scanning Electron Microscopy Evaluation

Scanning electron microscopy (SEM) provides in-depth information about the surface characteristics of a formulation. This has to do with how the constituents or particles in the formulations are made up, sized, and formed.[16] According to the SEM images, the structures were long and thin, with a fairly constant diameter throughout (Figure 1). The image was found to have a perfectly smooth, irregular-free surface—perfect for nanofiber.

In-vitro Drug Release

The *in-vitro* diffusion of all nanofibers was studied. Results showed that, as concentration of polymers increases, %drug diffusion increases (Table 3). NF3 found maximum drug release and NF2 found minimum drug release (Figure 2). Studies showed that as a result, curcumin was released in a controlled manner from all formulations. Their degradation caused the dispersal of the drugs within the nanofibers. It was discovered that fish collagen, a polymer, is biodegradable.[17,18] Data from *in-vitro* drug release experiments were shown as cumulative drug release vs time to analyze the release kinetics.

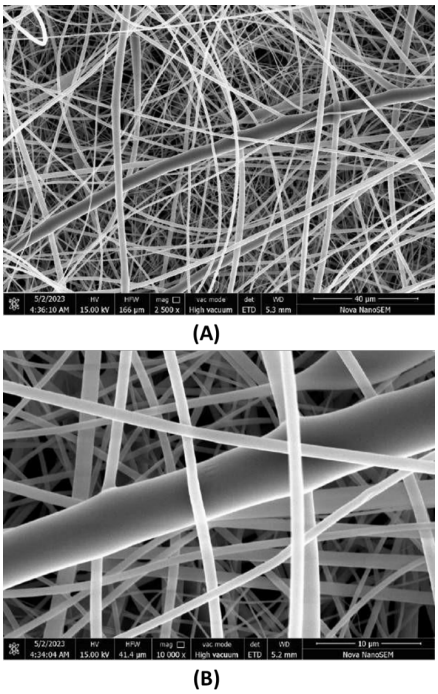

Figure 1: SEM representation of NF3 at magnification (A) 500X, (B) 2.5 KX

Table 3: *In-vitro* drug release profile

Time (hours)	*NF1 (%)*	*NF2 (%)*	*NF3 (%)*	*NF4 (%)*
0	0	0	0	0
0.5	11.43	12.62	19.77	12.66
1	27.28	20.73	32.33	25.71
2	42.77	34.19	51.89	39.04
3	39.95	60.11	65.74	53.72
4	70.99	71.02	86.39	77.86
5	80.32	75.93	97.71	73.09

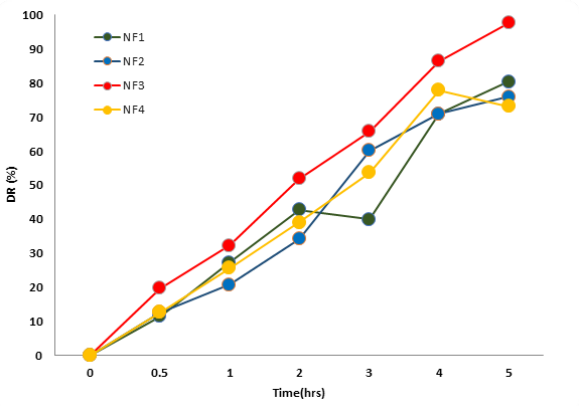

Figure 2: Graph showing *in-vitro* drug release of fabricated nanofibers

All formulations followed the zero order kinetic model required for transdermal preparations.

In-vivo Wound Healing

The extent of the wound gradually shrinks over time. The untreated rat exhibited the poorest rate of wound recovery, with an observed wound closure rate of 15.96 and 51.04% on days 5 and 10, respectively, while rat with blank nanofiber showed wound closer to 18.94 and 67.65%, respectively on days 5 and 10, Whereas rat with nanofiber with drug and polymer showed maximum wound closing percentage. It was observed to be around 31.54 and 89.65%, respectively on day 5 and 10. This concludes that the prepared nanofiber was proven to be effective in diabetic wound treatment as it causes angiogenesis and wounds closer rapidly.

CONCLUSION

In the current research, an effort was made to formulate nanofibers for diabetic wounds using *Boswellia* and polymers such as PCL and PVP. All the nanofibers were made and evaluated for further studies. Entrapment efficiency and swelling index were studied, and all nanofibers were effective for diabetic wound treatment. FTIR studies showed that prepared nanofiber has functional groups that are responsible for anti-inflammatory and angiogenetic properties. An *in-vitro* study was performed, and a graph was plotted. These studies found that nanofiber, NF3, showed maximum properties of ideal nanofiber for diabetic wound dressing. In the SEM study of formulation, NF3 was performed, which showed a uniform and smooth nanofiber surface without any irregularities. A wound closer study proved it to be effective in the treatment of diabetic wounds as it was observed to close wounds rapidly and effectively.

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