

# Original Article

## Fabrication of Polyvinyl Alcohol/Kefiran Nanofibers Membrane Using Electrospinning

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### Abstract

The Poly (vinyl alcohol)/Kefirane nanofiber membrane was successfully fabricated for the first time using electrospinning of the polyvinyl alcohol (PVA) and Kefirane blend solution. Scanning electron microscope (SEM), attenuated total reflectance Fourier transform infrared (ATR FT-IR), and differential scanning calorimetry (DSC) were used to characterize the electrospun Poly (vinyl alcohol)/Kefirane fiber membranes. Similar analysis was done for polyvinyl alcohol nanofibers. Then, the results were compared with each other. Rheological behavior of the Poly (vinyl alcohol)/Kefirane solution was evaluated using a cone and plate rheometer. The flow curves of 6% Kefiran solution and Poly (vinyl alcohol)//Kefiran blend solution showed sufficient viscosity for electrospinning. Viscosity decreased as the shear rate has increased in two samples, with both demonstrating shears thinning behavior. Electrospinnability of Poly (vinyl alcohol)/Kefiran solution at different process conditions and Kefiran concentrations was studied. Scanning electron microscope analysis exhibited that diameter of polyvinyl alcohol nanofibers increased with increasing Kefiran. Increasing the Kefiran concentration to 6% led to the disappearance of nodes in nanofibers. Attenuated total reflectance Fourier transform infrared spectra showed that Kefiran has maintained its molecular structure during the electrospinning process. Differential scanning calorimetry of Poly (vinyl alcohol)/Kefirane nanofibers revealed lower melting point than polyvinyl alcohol nanofibers.

**Keywords:** Polyvinyl alcohol, Kefiran, Electrospinning, Nanofiber, Membrane

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## Introduction

Nanofibers are important one-dimensional nanostructure. Electrospinning is a versatile method for the preparation of ultra-long, one-dimensional nanofibers. This method uses an electromagnetic field with a voltage sufficient to overcome the surface tension of a polymer solution and to draw it into an ultrathin nanofiber. This method is of great importance to prepare the nanofibers from different polymers, especially the production of biocompatible polymeric nanofibers such as polyglycolic acid, polycaprolactam, polyvinyl alcohol (PVA) and chitosan.

PVA is a water-soluble, biocompatible and biodegradable synthetic polymer. This inexpensive and non-toxic polymer is a good candidate to use in the membrane, medical device, coating, textile and drug delivery, because of good physical properties, high hydrophilicity, processability, and good chemical resistance. Electrospun PVA nanofiber membrane has been commercialized since the 1950s. These membranes are freestanding, non-soluble in many solvents with high surface to volume ratio which makes it suitable for use in various fields. PVA membrane containing biologically active compound is more favorable for biomedical application than the pure PVA membrane. Scaffolds of PVA/collagen nanofibers have shown high efficiency in bone tissue engineering. PVA/chitosan, PVA/Maleic anhydride, and PVA/Keratin blend nanofibers have been produced by electrospinning. However, to best of our knowledge, there has been no report on electrospinning of PVA/Kefiran nanofibers.

Kefiran is exopolysaccharide that can be found in kefir grains. When kefir grains cultured in deproteinized whey, Kefiran is produced. This water-soluble biopolymer has good antibacterial, antifungal, and antitumor activity. It is expected that the membrane composed of nanofibers of PVA and kefir blend produced by electrospinning will play an important role in the biomedical field because of medicinal properties of kefir besides the good physicochemical properties of PVA.

In this study, kefir was extracted from cultured kefir grains. Different concentration of homogenous kefir solutions was mixed with 8% PVA solution followed by electrospinning. Rheological behavior of the PVA/Kf solution was evaluated using a cone and plate rheometer. The morphology, structure and thermal behavior of electrospun membranes were characterized by scanning electron microscope (SEM), attenuated total reflectance Fourier transform infrared (FT-IR) and differential scanning calorimeter (DSC), respectively.

## Materials and Methods

PVA powder ( $M_w=88,000 \text{ g mol}^{-1}$ , 88% hydrolyzed) was purchased from Sigma. Reagents were used without further purification and deionized water was used as a solvent.

8% PVA solutions were prepared from PVA powder and distilled water. 0.8 gr PVA powder was added into 100 mL distilled water slowly at room temperature. The mixture was stirred at 80 °C for 2 hours to get homogenous solutions.

Kefir grains, abundantly found and used in households in Iran, was used as a starter culture in this study. 100 g of the grains were cultured in skimmed milk in a plastic container with a closed sealed door at room temperature overnight, and the medium was exchanged daily for a new culture. These processes were continued for 7 subsequent days in order to consider the grains active. During the process, the container was covered with a coating to avoid direct light.

Kefiran biopolymers were extracted from kefir grains by the method reported previously. A weighed amount of cultured kefir grains was treated in boiling water (with a weight ratio of kefir grain to distilled water of 1:10) for 30 min with continuous stirring. The mixture was centrifuged at  $10,000 \times g$  for 20 min at 20 °C. The polysaccharide in the supernatant was precipitated by addition of two volumes of cold ethanol (96%; Merck) and left for overnight at -20 °C. The mixture was centrifuged at  $10,000 \times g$  for 20 min

at 4 °C. The obtained pellets were dissolved in hot water and the precipitation procedure was repeated twice. The precipitate was eventually dissolved in hot distilled water and heated to 60 °C in the oven to obtain dried Kefiran. Kefiran solution was prepared by dissolving in boiling water and being stirred at 90 °C. After the solution cooled down, PVA solution was added with vigorous stirring to get homogenous PVA/Kefiran solution. PVA/Kefiran solution was then electrospun immediately after being prepared.

The electrospinning process was carried out using Electroris (FNM Ltd., Iran, www.fnm.ir) as an electrospinner. The obtained solution was loaded into a plastic syringe with an inner diameter of pinhead of 0.80 mm. The syringe was connected to the device. In this experiment, a voltage of 20 kV was applied for electrospinning. The rate of flow was 1 ml/h and aluminum foil served as the counter electrode. The distance between the capillary and the substrate electrode was 12 cm.

The morphology of PVA and PVA/Kefiran membranes was observed by using SEM (Philips XL 30 and S-4160) with a gold coating. The rheological measurements of the Kefiran solutions were performed through an Anton Paar (PhysicaTM) MCR300 rheometer, using cone and plate geometry with 25 mm diameter for more and 50 mm diameter for a less concentrated solution (3.00 and 4.00 wt% solutions). The cone angle and the gap were set at 1° and 0.05 mm,

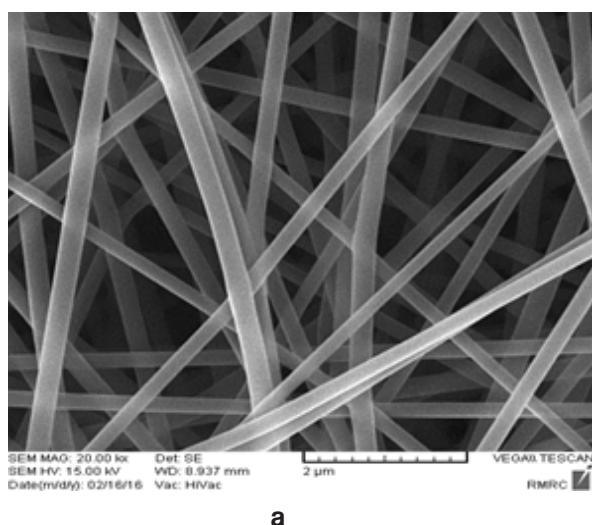
respectively. The shear viscosities were recorded in the shear rate ranging from 0.1 s<sup>-1</sup> to 100 s<sup>-1</sup> at 25 °C.

## Result and discussion

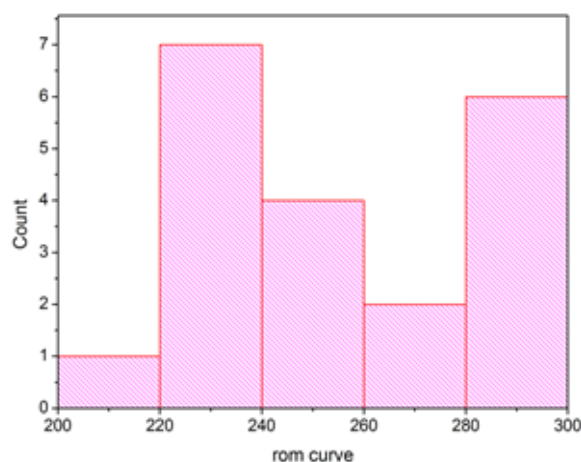
As the first step of the experiment, electrospinning of pure PVA was conducted. According to the previous study, 8% concentration of PVA in water has the best result in nanofiber production. Therefore, after preparation of 8% w/w PVA solution with water as a solvent, electrospinning of the polymer solution was carried out successfully. Electrospinning test set up included injector to collector distance of 120 mm, voltage of 12 kV, and injection rate at 1 ml/h.

As shown in Figure 1 uniform and continuous nanofiber was produced without any observed adhesion or knot. The average diameter of 20 nanofibers (calculated by measurement software) was 254 nm.

For the preparation of PVA/Kefiran blend, 4% w/w Kefiran solution was prepared in water as a solvent then mixed with PVA 8%. The mixing ratio of kefiran: PVA was 1:1. It was observed that the mixed solution was non-homogenous and unstable at room temperature and separation of polymer occurred. Therefore, 4% w/w tween 80 surfactants were added for preparation of



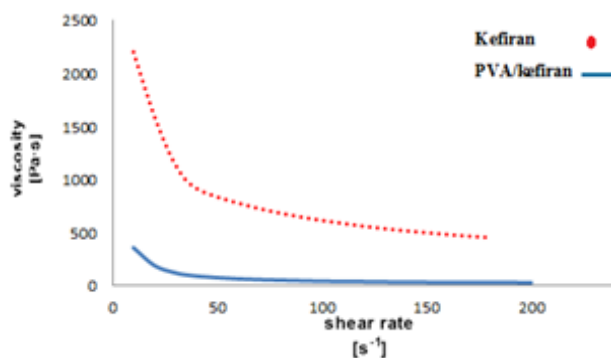
a



b

Figure1: a) SEM image of PVA nanofiber

b) fiber diameter distribution of PVA nanofiber



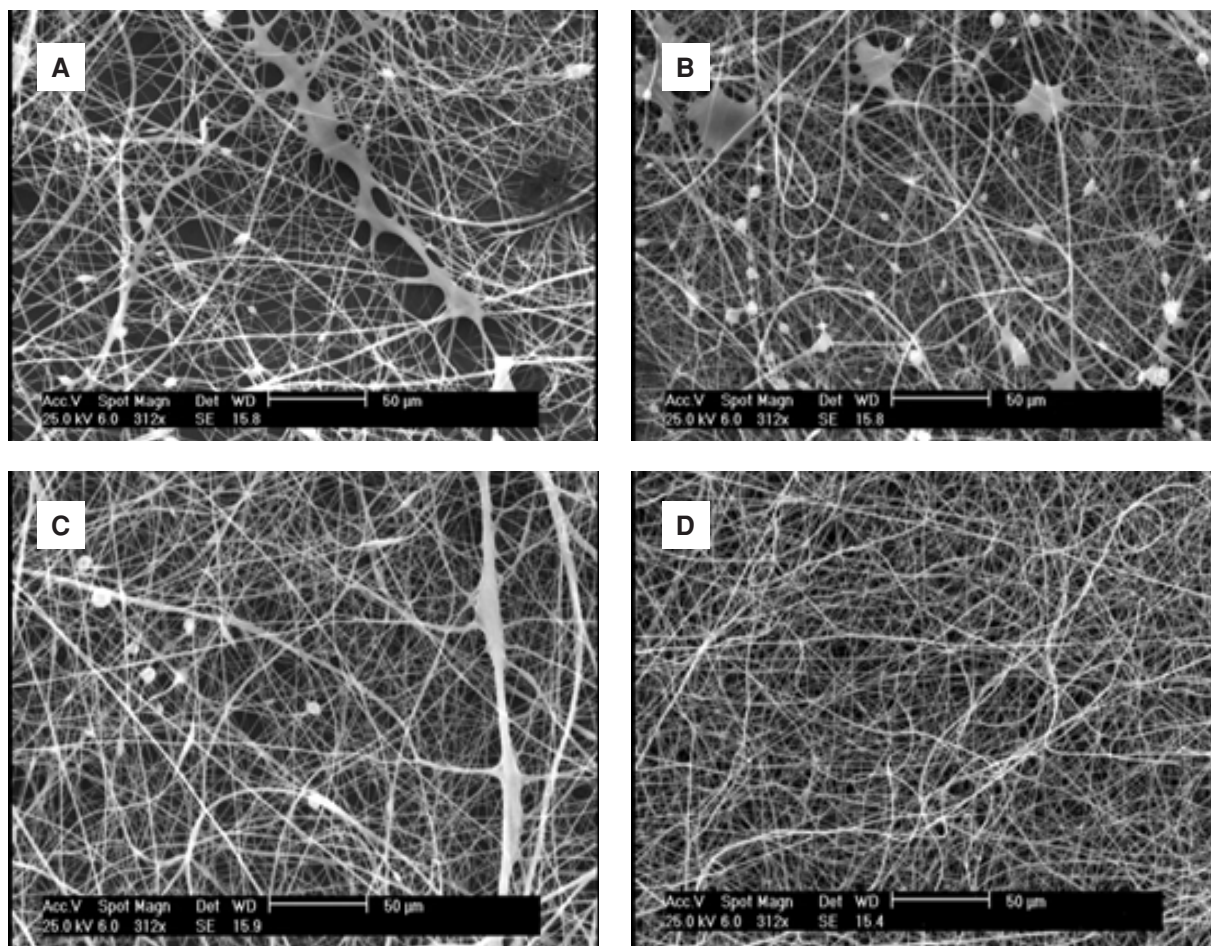
**Figure 2: The flow curves of kefiran and PVA/kefiran solutions at 25°C.**

homogenous solution with good viscosity. The flow curves of 6 % Kefiran solution and PVA/ Kefiran blend solution were depicted in Figure 2. Viscosity has decreased as the shear rate increased

in both samples, with both demonstrating shears thinning behavior, at the shear rate of 0.1–200 s<sup>-1</sup>. This trend is weak in PVA/Kefiran solution compared to Kefiran solution, although both have sufficient viscosity for electrospinning.

The morphology and diameter of electrospun nanofibers depend on the various parameters such as solution parameter (viscosity, concentration, PH, etc.) and process parameters (applied voltage, tip to collector distance, injection rate, etc.).

First, the electro spinnability of PVA/Kefiran solution in different process conditions was studied. For this purpose, 4% PVA/Kefiran solution was inserted into the syringe of Electror-is for electrospinning. Voltage, distance and injection rate varied by 12-20 V, 120-200 mm and 0.5-2 ml/h, respectively. In most conditions, electrospinning jet was not formed and nanofibers



**Figure 3: Influence of kefiran solution concentration on PVA/Kf nanofiber diameter; morphology of the nanofibers produced with (a) C = 3.00 wt% (b) C = 4.00 wt%, (c) C = 5.00, (d) C = 6.00 wt%, kefiran solution**

did not appear at room temperature. Increasing the temperature to 35 °C originated the appearance of Taylor cone of the nanofiber in some condition. Continuous electrospinning polymer drop on the drum occurred when the applied voltage, tip to collector distance, injection rate and temperature were selected at 15 KV, 150 mm, 2 ml/h and 35 °C, respectively.

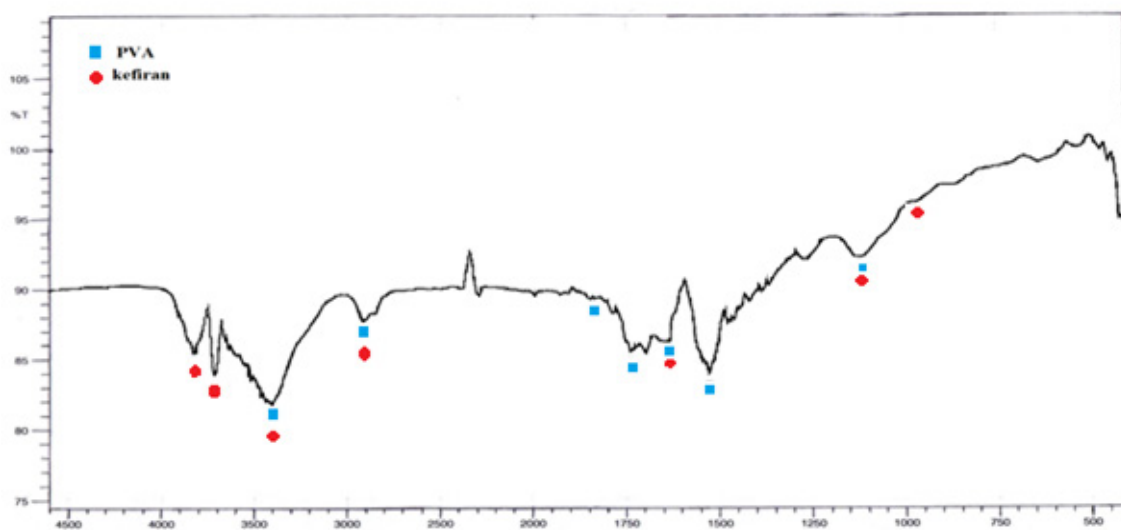
In order to achieve the appropriate concentration of Kefiran for electrospinning, different concentrations of Kefiran in water solution (3, 4, 5, 6 % w/w) were prepared then mixed with 8% PVA solution and 4% tween 80 surfactants. Blend weight ratio of polymers was fixed at 1:1 ratio in all solutions. The mixed polymers stirred for 2h to gain homogeneous solution then electrospun directly.

SEM images of electrospun PVA/Kefiran nanofiber with different concentrations of Kefiran are shown in Figure 3.

It is well known that from different solution

parameter, the polymer concentration which affects viscosity has a very important role in electrospinnability. Previous studies have found that low polymer concentration led to electrospraying instead of continuous nanofiber formation. It was observed that as the polymer concentration increased, the morphology of the collected mass changed from beads to gradually fine nanofibers. As shown in Figure 3, increasing the Kefiran concentration from 3 to 6 %, beads and nanofibers adhesion disappeared. Fine and continuous PVA/Kefiran nanofibers gained with 6% Kefiran concentration. The average diameter of fibers in PVA and PVA/Kefiran nanofibers is about 254 nm and 538 nm, respectively. Obviously, the latter average diameter is larger than the former. The blending of PVA and Kefiran during electrospinning enhances the solution viscosity thus, the average diameter of fibers in PVA/Kefiran membrane is larger.

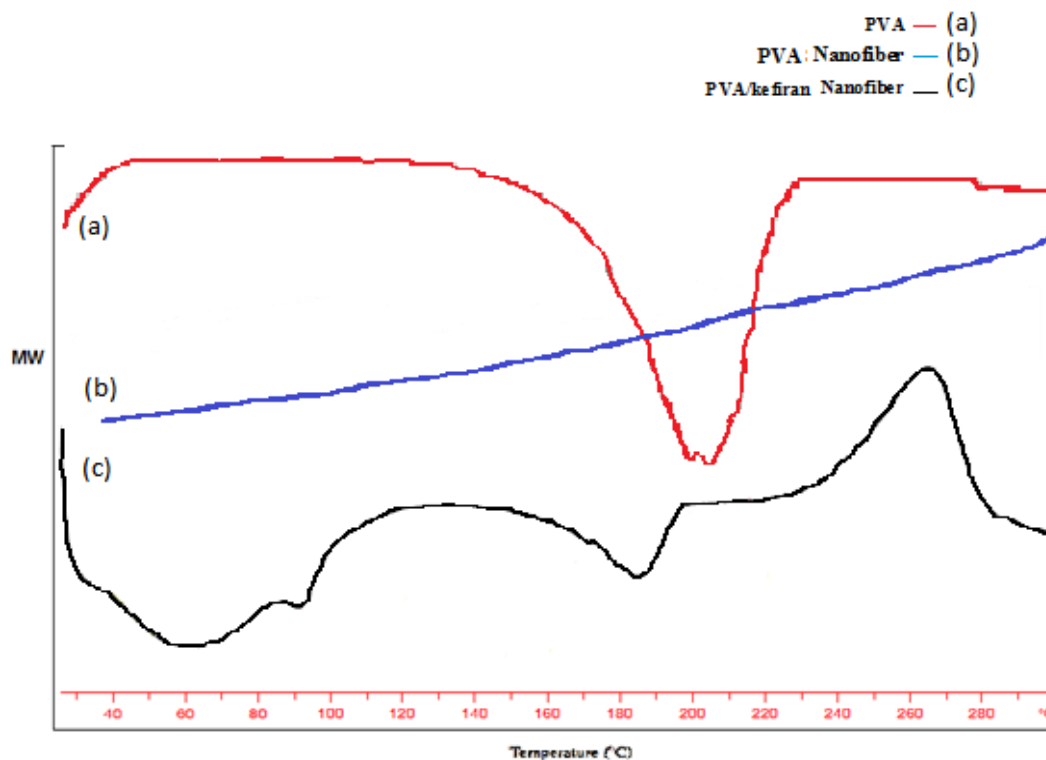
Figure 4 shows the FT-IR spectra of PVA/Kefiran



**Figure 4: FT-IR spectra of electrospun PVA/kefiran**

blend nanofibers. As shown in the figure, peaks comply with the structure of constituent. A number of absorptions peak at 2921, 2850, 1440, 1320, 1242, 1095, and 850  $\text{cm}^{-1}$ , which were attributed to the  $\text{—(CH}_2\text{)}$ ,  $\text{—(CH)}$ ,  $\text{—(CH—OH)}$ ,  $\text{—(CH—OH)}$ ,  $\text{—(CH)}$ ,  $\text{—(C—O)}$ , and  $\text{—(C—C)}$ . Broad rounded peaks above 3000  $\text{cm}^{-1}$  can be noticed which were indicative of different hydroxyl groups, related to polysaccharides and hydroxyl group of PVA. O-H bending mode of bound water also results in a peak at 1645  $\text{cm}^{-1}$ . The peaks in the region of 1300–1800  $\text{cm}^{-1}$  correspond to the bending and stretching vibrations of the PVA. The fingerprint region ranges from 1200–900  $\text{cm}^{-1}$  which associates with stretching mode

of carbohydrate rings and side groups (C-O-C, C-OH, and C-H). Moreover, the broad peak near 1100  $\text{cm}^{-1}$  is characteristic of the saccharide structure. Finally, the peaks in the vicinity of 900  $\text{cm}^{-1}$  are indicative of the vibration modes of glucose and galactose in the structure of pure Kefiran [22].



**Figure 5. DSC thermograms of a) pure PVA b) electrospun PVA nanofibers b) electrospun PVA/ Kefiran nanofibers.**

DSC thermograms of electrospun PVA and PVA/Kf membranes are shown in Figure 5. It is generally believed that the melting endothermic peak ( $T_m$ ) of PVA appears around 205 °C. It can be seen from thermogram of PVA/Kefiran nanofibers that clear melting endothermic peak appears around 185 °C, but there is no clear melting endothermic peak in thermogram of PVA nanofibers. Crystal structure was destroyed when PVA powder dissolved in water. High elongation rate and the rapid solidification process during the electrospinning results in a low degree of crystallinity and non-clear melting endothermic peak in PVA nanofibers. However, three-dimensional structural molecules and crystal structure were formed in electrospun PVA/Kefiran nanofibers. Consequently, clear melting endothermic peak was observed in thermogram of PVA/Kefiran nanofibers. The

melting point of PVA decreases with the addition of Kefiran. The glass-transition temperature ( $T_g$ ) and the degradation temperature of PVA/Kefiran nanofibers were measured at 54 °C and 266 °C, respectively.

## Conclusion

Polyvinyl alcohol/Kefiran nanofibers were fabricated from electrospinning of the polyvinyl alcohol and Kefirane (extracted from kefir seed) blend solution.

Rheological behavior of the Poly (vinyl alcohol)/Kefirane solution was evaluated using a cone and plate rheometer. The flow curves of 6% Kefiran solution and Poly (vinyl alcohol) /Kefiran blend

solution showed sufficient viscosity for electrospinning. Viscosity decreased as the shear rate was increased in two samples, with both demonstrating shears thinning behavior.

Characterization of the electrospun Poly (vinyl alcohol) /Kefiran fiber membranes was done by SEM, ATR FT-IR, and DSC. SEM analysis exhibited that diameters of PVA nanofibers increased with increasing Kefiran. Increasing the Kefiran concentration to 6% led to the disappearance of the nodes in nanofibers. ATR-FTIR spectra showed that Kefiran has maintained its molecular structure during the electrospinning process. DSC of Poly (vinyl alcohol)/Kefiran nanofibers showed lower melting point than Poly (vinyl alcohol) nanofibers.

## Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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