

## Fabrication and investigation of antibacterial properties of modified polyvinyl alcohol nanofibers containing Mummy

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### Abstract

**Purpose:** Polymer nanofibers are nanostructures with many applications in medical science. Electrospinning is a simple method to produce fibers with different thicknesses. Mummy is a drug with anti-inflammatory, antioxidant, and anti-cancer properties. Therefore, in this study, we synthesized nanofibers modified from PVA together with Mummy by electrospinning process, then we investigated their antibacterial properties.

**Method:** First, the Mummy material was soaked. Then, nanofibers were prepared with PVA by electrospinning method. The morphological and structural properties of nanofibers were analyzed by scanning microscope (SEM) and Fourier transform infrared spectrometer (FT-IR) techniques, and their antimicrobial properties against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were investigated.

**Results:** The results of SEM analysis showed that nanofibers were prepared with an average diameter of 329.5 and 444.3 nm in the presence and absence of Mummy substance, respectively. The produced nanofibers containing Mummy had inhibitory properties for both the bacteria used, but it had the most antimicrobial properties against Gram-negative bacteria *Pseudomonas aeruginosa*.

**Conclusion:** The results of this research showed that it is possible to load the Mummy substance on PVA nanofibers in order to produce an active substrate with antioxidant and antibacterial properties, and it improves the physical properties of nanofibers.

**Keywords:** Modified nanofibers, electrospinning, polyvinyl alcohol, Mummy, antibacterial



## Introduction

Nanotechnology refers to the use of materials in atomic, molecular and supramolecular dimensions in industrial applications[1]. Electrospinning is known as one of the famous methods of fiber production in scientific research of various materials. This nanofibrous structure has the important feature of large surface to volume, high porosity and superior mechanical properties[3,2]. The importance of biodegradable and biocompatible polymers such as polyvinyl alcohol (PVA), alginate, starch and chitosan or their derivatives has increased significantly in the last two decades due to their reversible and acceptable biological properties[4]. Polyvinyl alcohol is a hydrophilic synthetic polymer. In addition to its hydrophilicity, its advantages include biocompatibility, non-toxicity, high water content, strong mechanical properties and high chemical stability compared to other synthetic polymers[5]. Medicinal plants and natural substances have been used to treat various diseases for thousands of years. Even today, traditional medicine is very popular among people and many people use these substances to treat diseases[6]. Mummy (Shilajit) is one of the old medicines of Iran and is a black or brown semi-solid substance that is produced by the oxidation of petroleum hydrocarbons in the cracks of high mountains[7]. Mummy, a traditional Iranian medicine, has been used for hundreds of years to heal joint inflammation, bone fractures, and wound healing. The color of this material is dark brown to black, which is the result of oil oxidation in the cracks of some caves. Mummy contains various ions such as phosphate, calcium, hydrocarbons, polysaccharides and nitrogen. Recently, some researchers have investigated the potential effects of Mummy in healing skin wounds, stomach ulcers, and bone fractures[8]. Mummy is a medicine with anti-inflammatory, antioxidant and anti-cancer properties[9]. Considering the antioxidant effects of the above medicinal substance, therefore, in the present study, we decided to first synthesize modified polyvinyl alcohol nanofibers using Mummy, then to investigate their antibacterial properties on Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus*.

## Materials and methods:

### Preparation of polyvinyl alcohol polymer solution containing Mummy:

Mummy material was carefully washed using distilled water. Then, 0.5 g of Mummy was placed in an Erlenmeyer flask and 5 ml of hydroalcoholic solvent (3:7 ratio of water and ethanol) was added to it. First, Erlen was placed in an ultrasonic device for 10 minutes, and then on a magnetic stirrer for about an hour, in a dark environment for 72 hours. After 72 hours, the Erlen content was filtered and separated from the solvent.

PVA polymer was dissolved in distilled water by a magnetic stirrer at 80 °C to produce a 10% PVA solution (by mass). The Mummy was added to the PVA solution. Then, it was stirred for 3 hours at room temperature to obtain a viscous gel. In the final stage, the obtained gel was prepared for injection into the electrospinning machine.

To produce nanofibers, the polymer solution prepared in the electrospinning process was spun under a voltage of 25 kV, at a distance of 10 cm and with a flow rate of 0.3 ml/hour, the polymer solution containing the Mummy substance was spun for two hours.

In this research, to investigate the chemical structure of nanofibers and connections between Mummy extract and polymer, Fourier transform infrared spectroscopic analysis (FT-IR) was used in the range of cm<sup>-1</sup> 1500-4000 with (Tensor II device model and made by Bruker, Germany). Also, in order to investigate the morphology of modified polyvinyl alcohol polymer nanofibers and the diameter of nanofibers, a scanning electron microscope (TESCAN MIRA4 device model and manufactured by TESCAN company) was used, and images were prepared with a magnification of 300,000 times.

### Antimicrobial tests:

Two types of gram-negative bacteria *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus* were chosen as model bacteria. All bacteria were grown in food broth medium and kept in Nutrite agar at 4°C until use.

After the bacterial culture, a suspension was prepared from it and using the standard solution of Neem McFarland, the bacterial cells were homogeneously spread on the agar plates with a sterilized swab. Nanofibers containing Mummy, Mummy extract and nanofibers were placed under UV lamp for 20 minutes to sterilize. Each sample was placed on an agar plate. The samples and inoculated agar plates were incubated at 37 degrees for 24 hours. Then, the antibacterial activity of nanofibers containing Mummy substance was determined after three repetitions and the average results were reported.

### Data analysis methods:

One Way ANOVA statistical test was used in order to investigate the significant difference between the average diameter of the halo of non-growth of bacteria for extract, Mummy nanofibers and nanofibers without extract. The significance limit was considered as p-value < 0.05.

## Results and discussion:

### scanning electron microscope (SEM):

Figure1 - SEM images of PVA nanofibers and modified nanofibers containing Mummy with a concentration of 10% PVA (by mass). As can be seen, the pure PVA nanofibers and Mummy-modified nanofibers with a concentration of 10% PVA (by mass) show a smooth morphology with an average diameter of 329.5 nm and 444.3 nm, respectively. On the other hand, it can be seen that by adding the desired material, the diameter of the fibers increases [10].

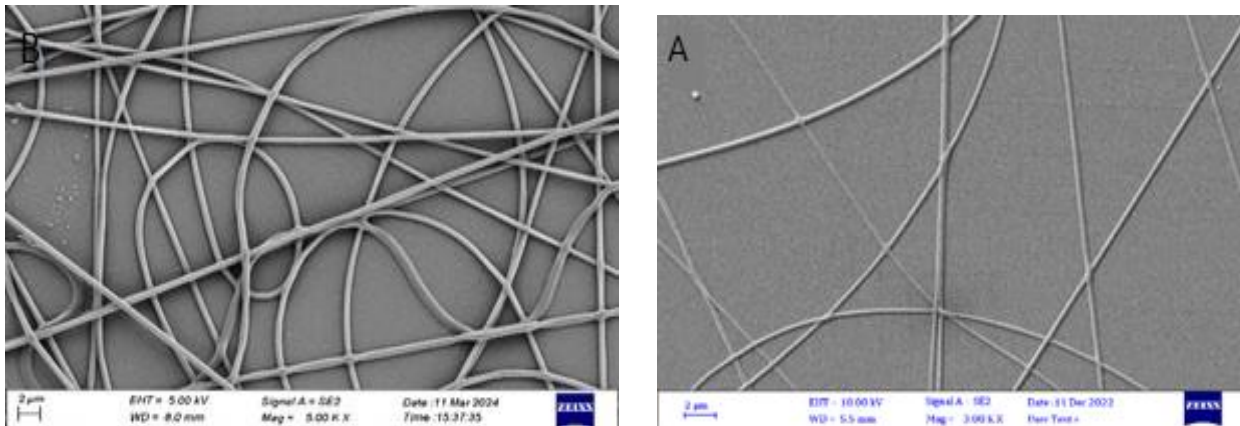


Figure 1-SEM of polyvinyl alcohol nanofibers (A) and modified polyvinyl alcohol nanofibers containing Mummy 10 (%w/V) (B)

### Fourier transform infrared spectroscopy (FT-IR):

FT-IR spectrum was used in order to investigate the functional groups and bonds in the raw materials as well as the bonds made from polyvinyl alcohol nanofibers and polyvinyl alcohol nanofibers modified with Mummy.

Figures (2-2) and (2-3) show the FT-IR spectrum for polyvinyl alcohol nanofibers and polyvinyl alcohol nanofibers modified with mummy, respectively, in the range of 400 and 4000  $\text{cm}^{-1}$ . A strong peak in the range of 3450/71  $\text{cm}^{-1}$  is related to O-H stretching vibrations, which is considered the most important feature of alcohols. For figure (2-2), respectively, the peaks around 2890.24  $\text{cm}^{-1}$  and 2978.79  $\text{cm}^{-1}$  correspond to the stretching vibration of the C-H bond. The presence of a strong peak in the area of 2924-2875  $\text{cm}^{-1}$ , respectively, of the symmetric and asymmetric stretching bands of  $\text{CH}_2$ , as well as the presence of three peaks in the areas of 1438/123  $\text{cm}^{-1}$ , 1379/73  $\text{cm}^{-1}$ , and 1639/196  $\text{cm}^{-1}$ , respectively, related to  $\text{CH}_3$  - Bending and  $\text{CH}_2$ -stretching, in addition to this, the presence of a peak at 1459  $\text{cm}^{-1}$  is due to the C-C band and the presence of a peak in the area of 1350-1500  $\text{cm}^{-1}$  is acetate, this area is due to the stretching of the C-O bond.

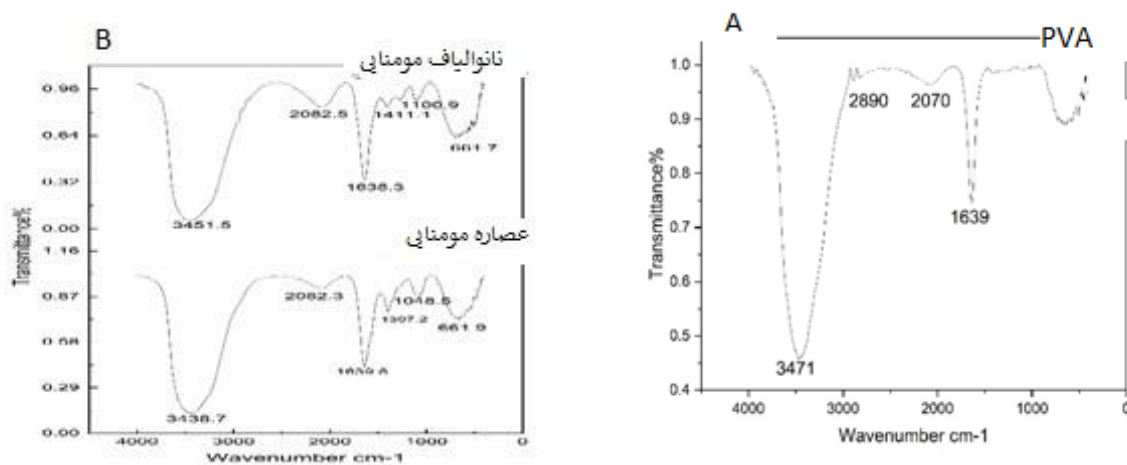
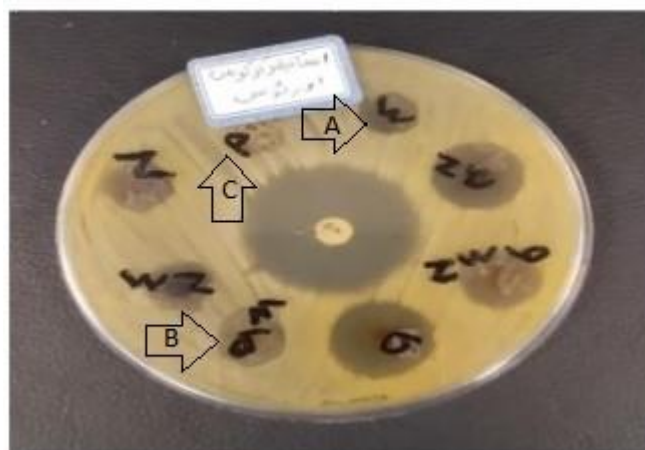


Figure 2- FTIR spectrum for polyvinyl alcohol nanofibers (A) and modified polyvinyl alcohol nanofibers containing Mummy (B) respectively

### Antibacterial test:

In the present study, the antibacterial activity of mumnai extract, PVA nanofibers modified with Mummy substance and PVA nanofibers was investigated by measuring the halo of non-growth for both Gram-positive and Gram-negative bacteria.

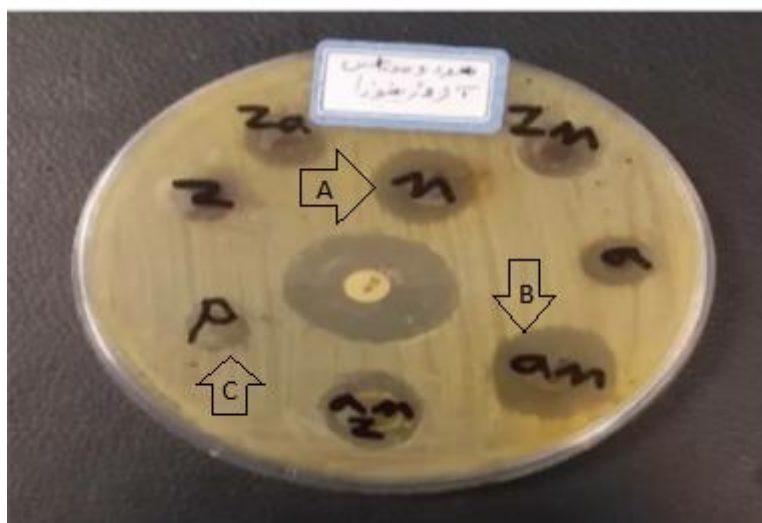


**Figure 3- Antibacterial activity of Mummy extract (A), PVA nanofibers modified with Mummy (B) and PVA nanofibers (C) against *S. aureus* bacteria in a period of 24 hours**

As can be seen in table (1), the average halo of non-growth of *Staphylococcus aureus* bacteria in Mummy extract is 7.5 mm, in modified nanofibers containing Mummy is 15.5 mm, and in poly polymer nanofibers. Vinyl alcohol formed a negligible growth halo. Clindamycin antibiogram disk was used for positive control.

**Table 1- Investigating the average halo of non-growth of *Staphylococcus aureus* bacteria in millimeters for nanofibers containing Mummy**

| row | Material name    | No growth of bacteria the second time | No growth of bacteria the first time | No growth of bacteria the third time | average |
|-----|------------------|---------------------------------------|--------------------------------------|--------------------------------------|---------|
| 1   | Mummy            | 8                                     | 7                                    | 8                                    | 7/5     |
| 2   | Mummy nanofibers | 15                                    | 16                                   | 16                                   | 15/5    |
| 3   | PVA polymer      | 1                                     | 1                                    | 1                                    | 1       |
| 4   | Positive control | 25                                    | 25                                   | 25                                   | 25      |
| 5   | Negative control | -                                     | -                                    | -                                    | -       |



**Figure 4- Antibacterial activity of Mummy extract (A), Mummy -modified PVA nanofibers (B) and PVA nanofibers (C) against *P. aeruginosa* bacteria in a period of 24 hours**

As shown in figure (4) and table (2); The average halo of non-growth in *Pseudomonas aeruginosa* bacteria, related to Mummy extract was 9.5 mm and in nanofibers modified with Mummy was 17.5 mm. Also, polyvinyl alcohol nanofiber showed the lowest non-growth halo, with an average of 1. Azithromycin antibiogram disc was used for positive control.

**Table 2- Investigating the average aura of non-growth of *Pseudomonas aeruginosa* bacteria in millimeters for nanofibers containing Mummy**

| row | Material name    | No growth of bacteria the second time | No growth of bacteria the first time | No growth of bacteria the third time | average |
|-----|------------------|---------------------------------------|--------------------------------------|--------------------------------------|---------|
| 1   | Mummy            | 9                                     | 10                                   | 10                                   | 9/5     |
| 2   | Mummy nanofibers | 18                                    | 17                                   | 18                                   | 17/5    |
| 3   | PVA polymer      | 1                                     | 1                                    | 1                                    | 1       |
| 4   | Positive control | 25                                    | 25                                   | 25                                   | 25      |
| 5   | Negative control | -                                     | -                                    | -                                    | -       |

Usually, the antimicrobial activity of extracts is caused by free hydroxyls, which have the ability to combine with carbohydrates and proteins in the bacterial cell wall and can release antimicrobial agents when they come in contact with bacteria [11]. Also, Momenai nanofibers had the least effect on the Gram-positive *Staphylococcus aureus* bacteria and the most effect on the Gram-negative *Pseudomonas aeruginosa* bacteria. The observed difference can probably be due to the type and amount of effective compounds in the modified nanofibers, which are humic acid and folic acid compounds in the Mummy [12] Therefore, *Staphylococcus aureus* is a gram-positive bacterium and has a thicker cell wall, so it shows more resistance to antibacterial substances than the gram-negative bacterium *Pseudomonas aeruginosa* [13].



### Conclusion:

In this research, polyvinyl alcohol solutions modified with Mummy were subjected to electrospinning process to produce nanofibers. The results showed that nanofibers containing Mummy had a significant effect on the diameter and structure of nanofibers, and a uniform structure was obtained without defects of beads and without willows. Mummy nanofibers had a greater non-growth halo against both gram-positive and negative bacteria than Mummy extract, although the most antimicrobial property was against Gram-negative *Pseudomonas aeruginosa*.

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