



## Nanotechnological Dressings Containing Pomegranate

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Electrospinning, which is a nanotechnological method for rapid healing of wounds of diabetics, is aimed at naturally existing wound dressings. Polyvinyl alcohol (PVA), which is very useful, has been used for the electrospinning method, which includes properties such as being easily soluble in water, easily rotated, and forming permanent nanofiber surfaces. A mixture of 10% by mass of pure water and granular PVA was prepared by weighing on a precision scale. The mixture was stirred in a heated magnetic stirrer for 3 hours up to 80 °C and left at room temperature for 24 hours. To the remaining 10% PVA, 5% potency pomegranate (PP) additive was added and mixed in a magnetic stirrer at 30 °C for 45 minutes. Body wounds need calories, protein, vitamins A-C and zinc to heal. PVA, PVA-PP composites have been successfully obtained with the difference of nanotechnology without allowing possible welding formations by electrospinning technique.

**Keywords:** Diabetes, polyvinyl alcohol, potency pomegranate, electrospinning, wound healing

SubmissionDate:08 March 2023

Acceptance Date: 10 June 2023

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### 1. Introduction

Wound dressings are medical textile products that help wounds heal quickly in a healthy environment and conditions [1]. Textile materials called medical (medical) textiles or Medtech have shown a rapid development in the textile industry recently. Medical textiles are textile materials produced or designed for medical conditions. Surgical threads, dressings, dressings, bandages, artificial organs and similar materials are among

medical textile products [2]. Medical textiles are textile materials designed to be widely used in all

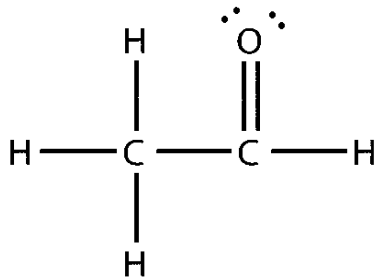
fields of medicine and surgery. Medical textiles should show biological compatibility with the surrounding tissue and combine with different materials. If we list the general properties required from medical textiles depending on the usage area; strength, antitoxic, elasticity, antiallergic, durability and biocompatibility. Biomedical materials can be contaminated with bacteria, and at this point, sterilization of biomedical materials is also very important and one of the factors to be considered [3].

Bitter melon (*Momordica charantia*) (PP) is a plant species of cucurbitaceae (*Cucurbitaceae*) family that grows in tropical climate regions and is used as food and medicine. The fruit and leaves of the mighty pomegranate, which is a

medicinal plant used especially in the treatment of digestive system diseases and wounds, are very rich in minerals and vitamins, especially iron, calcium, phosphorus and B vitamins. There are a limited number of studies on the nutrient requirement of this plant, which is grown in the Aegean and Mediterranean regions of our country.

The fruit, which is very bitter in all parts and juice, is green when raw, orange-red when ripe, 10-20 cm long, in the form of a large shuttle. It has an indented, protruding and rough surface. When ripe, the husk is bent back to reveal numerous red, brown or white seeds. Its seeds (seeds) are 7-10 mm long, flat and rough. *Momordica Charantia*, which has a wide range of uses in recent years, is also suitable for local use such as wound healing and burn treatment, leading to great developments in medicine and medical sector.

Polyvinyl alcohol (PVA), a biocompatible and water-soluble hydroxy polymer, has very good chemical resistance, flexibility, mechanical strength and biodegradability. It has very good physical and mechanical properties, chemical stability at room temperature, and the ability to form very good fibrous materials alone or mixed with other polymers [5]. The chemical structure of PVA is shown in Figure 1.1.



**Figure 1.1.** PVA chemical structure [6]

In this study, materials that can show wound healing tape properties were produced from PVA-PP composites by electrospinning method. Characterization studies such as morphological (FEGSEM, Scanning Electron Microscopy with Field Emission Gun), biological (Antibacterial Test) and mechanical (Tensile Test) of these produced nano biocomposites were carried out. When the results of the study are evaluated, it will be an ideal material that can exhibit band-aid properties for the healing of foot wounds of diabetic diseases.

## 2. Material and Method

### 2.1. Materials

The bitter melon plant was obtained from herbalists and plant breeders around us. PVA molecular weight 85,000-124,000 g/mol (87-89% purity Sigma-Aldrich/Turkey), pure water

(Sigma-Aldrich/Turkey) to dissolve polymers and loiled paper as backing material in electrospinning method, in electrospinning step, Different size plastic syringes (Inyekta, Istanbul, Turkey) were used. The Sigma-Aldrich brand was chosen as the reference.

### 2.2. Nano biocomposite production

#### 2.2.1. Creation of nano biocomposite solutions

Polymer/PP solutions at different concentrations (w/v) were prepared for nano biocomposite production in the solvent system. According to the results obtained as a result of the preliminary trials, the process of preparing the polymer and polymer/PP solutions, respectively, is as follows; Electrospinning solution was prepared by adding 10 g of PVA by weight to 90 ml of distilled water stock solution, depending on the temperature and mixing times in Table 2.1. Different concentrations of 1 ml, 5 ml, 8 ml PP were added to the same PVA solution. The resulting solution was rested for 7 hours, and it was made ready for electrospinning by removing the bubbles it contained. In the studies carried out so far, nanofiber formation has become difficult when the amount of reinforcement in biocomposite production is above 8 ml. Over 8 ml, agglomeration problems and fiber structures can go from nano level to micron level and an irregular fiber structure occurs. For this reason, supplementation rates were determined as 1 ml, 5 ml, 8 ml [6]. Figure 2.1 shows the PP plant. Preparation of necessary solutions for nano biocomposite production is given in Table 2.1.



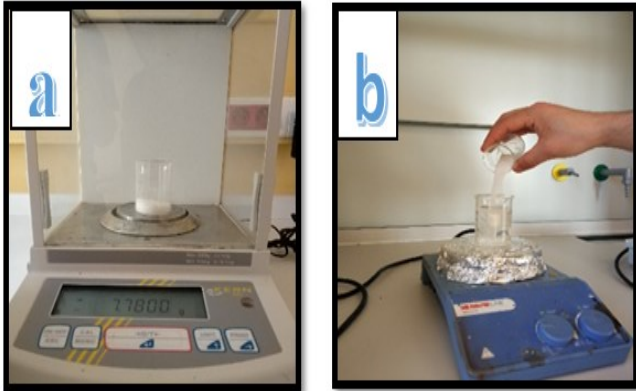
**Figure 2.1.** PP plant image

**Table 2.1:** Preparation of necessary solutions for nano biocomposite production

Polymer / Additive	Mixture ratio (w/v)	Mixture temperature (°C)	Mixing time (minute)
10 % PVA	9:1	30	180

10 % PVA- 1 ml PP	9:1	30	180
10 % PVA - 5 ml PP	9:1	35	190
10 % PVA- 8 ml PP	9:1	37	200

Preparation of necessary solutions for nano biocomposite production is given in Figure 2.2.



**Figure 2.2.** Preparation of necessary solutions for nano biocomposite production

### 2.3. Band-aid production by electrospinning technique



**Figure 2.3.** Electrospinning device used in the production of nano biocomposites (Inovenso NS 24XP, Turkey)

Using Inovenso brand (NS24XP, Turkey) electrospinning device, nanofibers were obtained by depositing them on a

collector oil paper at room temperature. The electrospinning device used in the production of nano biocomposites is given in Figure 2.3. The electrospinning parameters required for nano biocomposite production are shown in Table 2.2.

**Table 2.2:** Electrospinning parameters required for nano biocomposite fabrication

Polymer Additive	Working Distance (cm)	Flow Rate(ml/hour)	Voltage (kV)
10 % PVA	15	3.5	25.8
10 % PVA- 1 ml PP	15	3.5	25.8
10 % PVA- 5 ml PP	15	5	30.0
10 % PVA- 5 ml PP	15	5	30.0

Suitable solvent/solvent systems for the electrospinning process were investigated. Pure water was used in PVA and 1 ml, 5 ml, 8 ml PP added PVA solutions.

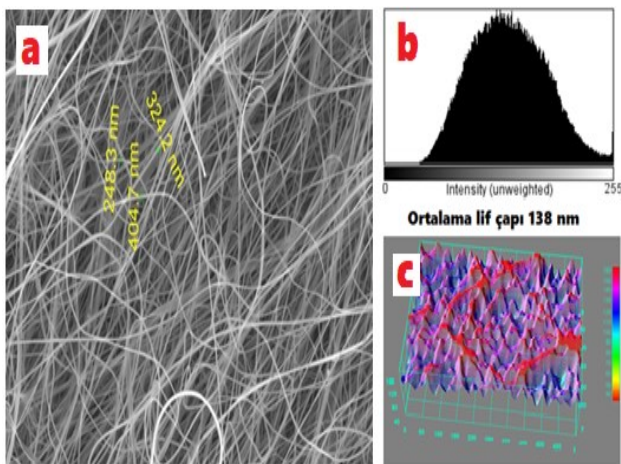
### 2.4. Nano biocomposite characterization studies

During the examination of the diameter and dimensions of the produced composite nanofibers, the images magnified at x6000 times were examined at 7 kV potential for FEGSEM analyses. The surface morphologies of the biocomposite nanofibers were determined in the measurements made in the FEI FEG QUANTA 450 brand device. The average diameter thickness of the formed nanofibers was measured with Image j (National Health Organization) software on high resolution FEGSEM photographs. The antimicrobial activity of electrospun mats containing different amounts of PP (1 ml, 5 ml, 8 ml) was investigated by the disc diffusion method and colony counting method according to the AATCC 100 method [7]. Antimicrobial activities were evaluated against Gram-positive (*Staphylococcus aureus* (ATCC 29213)) and Gram-negative (*Escherichia coli* (ATCC 25922)) bacteria. Strains were provided by Istanbul University Faculty of Veterinary Medicine, Department of Microbiology Culture Collection. For the qualitative measurement of antimicrobial activity, the disk diffusion method was used to screen the film samples. The films were cut into 1x1 mm<sup>2</sup> squares. Muller Hinton Agar plates were inoculated with 0.1 ml of 0.5 McFarland dilution of the tested culture. Film samples were placed on the surface of the inoculated plates. Plates were incubated at 37 °C for 24 hours. Plates were examined for possible open areas after incubation. The presence of any open zone formed on the plate medium around the film was noted as a zone of growth

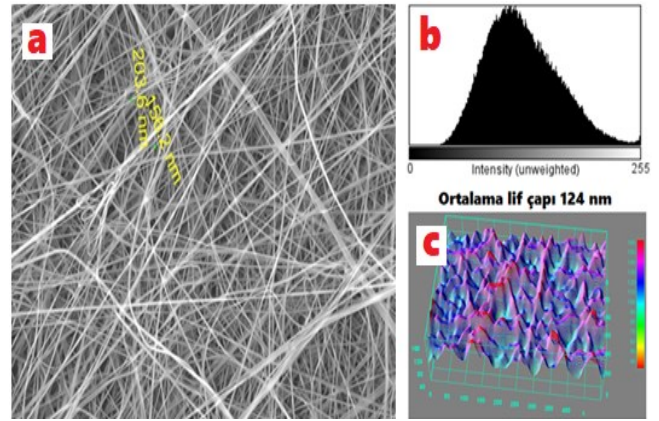
inhibition against microbial species. For positive control, gentamicin standard disc (10  $\mu\text{g}$ ) was placed on agar plates. The cultures used in the colony count method were 105 CFU/ml. In this procedure, Luria / Nutrient broth solution is prepared for bacterial inoculation with test and control samples in separate containers. The dishes containing the nutrient broth, samples and bacterial culture were shaken at 37 °C at 200 rpm for 24 hours. Agar plates were prepared by pouring the required amount of sterilized Luria/Nutrient agar and agar mixture into petri dishes and allowing it to solidify. After serial dilution, the growth liquid was spread over the surface of the solidified agar. All petri dishes were incubated at 37 °C for 24 hours. Total colony forming units were counted using a colony counter. In order to carry out the mechanical characterization studies of the biocomposites obtained after the electrospinning method, the samples were prepared in 1x5 cm dimensions [8]. A digital micrometer (795.1 MEXFL-25, Starrett, USA) device was used for thickness measurements of biocomposites cut to size. The resulting thickness values were entered into the analysis program before the mechanical analysis and used to determine the elastic modulus. The mechanical properties of the resulting nanofibers were determined with a Zwickline (Zwick/Roell Ltd., Germany) mechanical analyzer. The studies were carried out under room conditions. The samples were separated from the substrate and attached to the device clamps. The device is 5 mm<sup>2</sup>/min under 500 N load. The mechanical properties were determined with the device by adjusting the pulling speed and 10 mm jaw spacing [9].

### 3. Results and Discussion

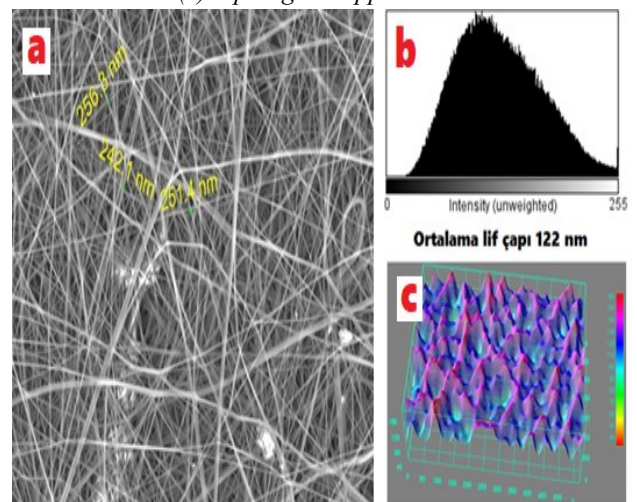
#### 3.1. Morphological analysis results



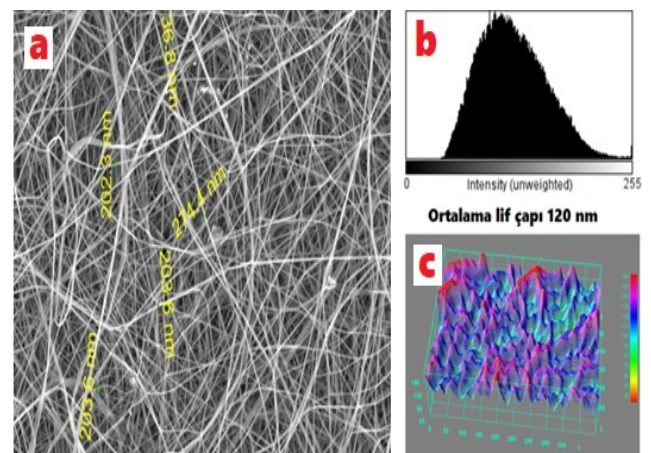
**Figure 3.1.** 10% PVA at X6000 magnification (a) morphological structure, (b) average fiber diameters and (c) topological appearance



**Figure 3.2.** 10% PVA-1 ml PP at X6000 magnification (a) morphological structure, (b) average fiber diameters and (c) topological appearance



**Figure 3.3.** 10% PVA-5 ml PP at X6000 magnification (a) morphological structure, (b) average fiber diameters and (c) topological appearance

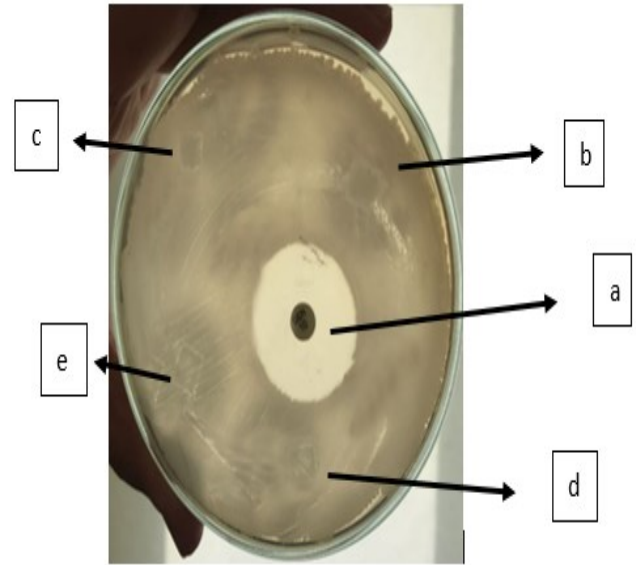


**Figure 3.4.** 10% PVA-8 ml PP at X6000 magnification (a) morphological structure, (b) average fiber diameters and (c) topological appearance

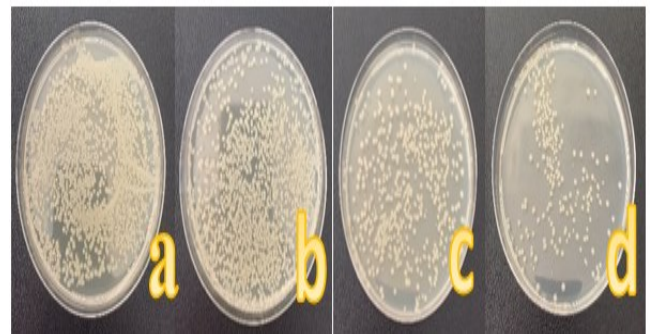
Figure 3.1., Figure 3.2., Figure 3.3. and Figure 3.4. shows the morphological structures of PVA and PVA-PP nano biocomposites. Nanofiber formation was observed in all samples. The fineness of nanofibers obtained by electrospinning from PVA/PP mixture solutions decreased as the amount of PP in the mixture increased. This is because; It is thought that with the increase of the PP ratio in the mixture, the solution viscosity decreases and the conductivity increases. The fiber diameters in the samples were measured with the FEGSEM device. When the dimensions of the nanofibers are examined, it is seen that the material produced in PVA/PP mixtures is formed as fine fibers. The PP additive wrapped the PVA polymer homogeneously without clumping. 10% PVA-8 ml PP sample is the composite with the thinnest fiber structure compared to other samples. It was determined by taking the average of the fibers measured in the device that the fiber average diameter was 120 nm [11]. As a result of FEGSEM morphological images, topological (surface distribution) images were extracted for all samples with Image J program. Thus, the surface distribution properties of the fibers in the internal structures of the samples were determined.

### 3.2. Antibacterial analysis results

The antimicrobial effect of electrospun mats containing different amounts of PP (1 ml, 5 ml, 8 ml) was qualitatively investigated with the zone of inhibition against *E. coli* and *S. aureus*, as shown in Figure 3.7. Clear sites of microbial inhibition for *E. coli* and *S. aureus* and completely surrounded by bacterial colonies. PP is a macromolecule and cannot be released into the bacterial environment. Therefore, we cannot observe a zone of inhibition. The antimicrobial effect of various PP-containing mats was investigated by colony counting method by comparing the number of bacterial viable cells. Figure 3.8. shows the antimicrobial activities of Electrospun mats by colony counting method. The number of viable cells was observed to be reduced in the electrospun mat containing 8 ml of PP compared to the control sample without PP. The mat containing 8 ml of PP showed 70% and 72% antimicrobial activity against *E. coli* and *S. aureus*, respectively. These results proved that the electrospun mat containing 8 ml of PP has antibacterial properties. Gupta et al. evaluated the antimicrobial properties of PP-loaded PVA/PEO/CMC coated polyester non-woven membranes in their study. They found that the membrane containing 10% PP showed 79.8% and 77.1% antimicrobial activity against *E. coli* and *S. aureus*, respectively. Therefore, they determined that PP loaded PVA/PEO/CMC coated polyester fabric membranes have excellent antimicrobial properties and are a promising biomaterial for wound dressing applications. In addition, the PP concentration (20%, 30%, 40% and 50% PP content of the membranes) increases, the antimicrobial activity of the membranes increases.

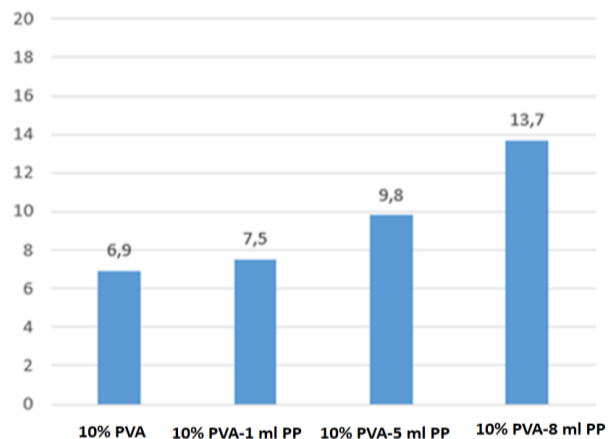


**Figure 3.5.** Antimicrobial activity of electrospun mats by disc diffusion method  
a) Gentamicin (control) b) PVA (control) c) 1 ml CH  
d) 5 ml AV e) 8 ml PP



**Figure 3.6.** Antimicrobial activity of electrospun mats by colony counting method;  
a) PVA (control) b) 1 ml PP c) 5 ml PP d) 8 ml PP

### 3.3. Tensile test results



**Figure 3.7.** Tensile test values of nano biocomposites

The strength values of the PVA/PP biocomposites in Figure 3.7. It was determined that the strength value increased linearly depending on the increase in the concentration percentages of the PP gel reinforced to the PVA matrix material [16,17]. As can be seen from the FEGSEM images we obtained as a result of morphological examinations, our mechanical properties have increased for these composites, in line with the results that PP is homogeneously coated on polymer fibers and no agglomeration formation is observed. Biocomposite materials have been produced with a strength much higher than the mechanical results found in the conjugate studies in the literature. This increase in strength is a result of both the polymeric matrix with high load carrying capacity, the PP additive and the homogeneous distribution in the composite [18-20].

#### 4. Conclusion

The fineness of the fibers obtained by electrospinning from PVA/PP mixed solutions decreased as the amount of PP in the mixture increased. This is because; It is thought that the solution viscosity decreases and conductivity increases with the increase in the PP ratio in the mixture. When we look at the fineness of the nanofibers in the nano biocomposite, it is seen that the nanofibers, which are a mixture of 10% PVA-8 ml PP, form the thinnest fibers. It is thought that the increase in the PVA ratio causes the thickness of the nanofibers to increase. In PVA/PP mixtures, it is thought that the increase in the PP ratio causes the increase in the charge density on the surface of the polymer jet extending in the attraction area created by the electrostatic forces, the jet showing more elongation and thinning of the fibers. Injection of the polymer through the needle during electrospinning causes the polymer concentration to decrease with the growth of molecular chains. Spinning parameters and solution properties also have an effect on this effect. Changing the solution parameters also increases this effect. In nanofibers and nano biocomposites in the amorphous structure formed by PVA polymer, an increase in T<sub>c</sub> glass transition temperatures was observed with the increase in the amount of PP in the structure. It is seen in the DSC thermograms that the increase in the amount of PP causes an increase in the thermal stability. It is thought that this thermal stability and the related increase in T<sub>c</sub> values are due to the cross-linking of PP and PVA polymer, which efficiently forms an amorphous structure. It is thought that with the increase in the amount of PP plant extract added to the PVA polymer, the amount of % crystallinity in the structure increases slightly and contributes to the mechanical properties of the obtained biocomposite materials. In this study, it was observed that the incorporation of PP contributed to the thermal and mechanical properties of nanofiber-containing composite structures. It has been determined that biocomposite nanofibers produced by antimicrobial test increase cell compatibility without creating any cytotoxic

effect. The tensile strength values of the biocomposite nanofibers produced by mechanical tests were determined and the highest tensile value was determined to be 13.7 MPa. When the obtained characterization results are evaluated, it can exhibit the feature of a band-aid that can be used as a wound healer for diabetic patients.

As a continuation of our work;

In the food packaging industry with its antimicrobial properties,

As a special wound dressing that we can control from the surface without touching the wound by providing wound healing,

In the treatment of some diseases that cannot be prevented by drug release,

In order to improve the strength properties after the work, in skin masks and cosmetic product groups of different polymers and chemicals, in wound-burn healing creams with PP additive,

Thanks to its antimicrobial properties, it is thought that it can be applied to the coating industry.

#### Acknowledgements

We would like to thank the Istanbul Arel University ArelPOTKAM team for their support in the realization of this study.

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