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The Effect of Curcumin Powder and Cloves Oil on the Properties of Fibers Produced by Electrospinning Technology

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Abstract

Electrospinning is a simple method for obtaining nanofibers. They possess distinctive properties such as large surface area to weight and high porosity, which make them attractive for many applications. In this research, the electrospinning technique was used to obtain non-woven mats of fibers using polylactic acid (PLA) with the addition of curcumin powder and clove oil as natural antibacterial materials. For the preparation of mats, a 10% polylactic acid solution was prepared in a mixture of acetone and dimethylformamide. Natural materials were added to the polymer solution in several concentrations of 1, 3, 5, 7, and 10%. The viscosity of these solutions was measured. The samples were then prepared using a locally manufactured electro-spinner. Using a scanning electron microscope, it was found that the average diameter of the fibers of the polylactic acid sample is 177 nm. It is noted that the diameters of the fibers produced using polymer and natural materials have larger diameters, especially when curcumin is added, as the fiber diameters range between 485 and 764 nm. The activity of the produced samples against two types of Gram-negative and Gram-positive bacteria was tested. It was found that the polymer-only sample did not have any resistance to the bacteria. While samples containing natural materials showed antibacterial activity against both types of bacteria. It was noted that this activity increased with the increase in the concentration of the additive, which produced an increase in the diameter of the inhibition zone, which makes it suitable for medical and other applications.

Keywords: antibacterial, Clove oil, Curcumin, Electrospinning, nanofiber, PolyLacticacide.

Introduction

Electrospinning is one of the most accessed nanofabrication techniques during the last three decades[1] . It is a simple route to generate polymer-based fibers with diameters on the nano-to-micron scale [2]. Nanofibers have a high surface-to-volume ratio and a porous structure [3]. Nanomaterials with eco-friendly properties are of interest to many researchers. Biopolymers have the advantages of biocompatibility, biodegradability, and non-toxicity, making them the most suitable polymers for use in medical fields [4]. PLA is a hydrophobic and biodegradable linear aliphatic polyester produced from renewable resources [3]. It plays an increasingly important role in medical applications owing to its unique properties as a Biopolymer [5]. Electrospun PLA nanofibers possess unique characteristics, such as similarity to the extracellular matrix, large specific surface area, high porosity with small pore size, and tunable mechanical properties. These fibers have recently led

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to advanced breakthroughs in the medical field, and are used for diverse applications [6]. PLA is a polymer with excellent mechanical properties, biodegradability, and biocompatibility, which makes it ideal for biomedical applications [7].

Antibiotic-resistant bacteria have become a global problem produced by the unprogrammed use of antibiotics, resulting in bacterial strains resistant to many antibiotics [8]. Nowadays, nanofibers with antimicrobial activity have a great interest because of the widespread antibiotic resistance of many pathogens[9]. Different antimicrobial agents are incorporated into electrospun nanofibers for the prevention of infection [10]. These agents include antibiotics, peptides, nanoparticles (e.g., titanium dioxide, silver, etc.), and natural substances (e.g., henna, chitosan, etc.)[11]. In medicine, the antimicrobial activity of silver nanoparticles is the ability to destroy a wide spectrum of pathogens and multidrug-resistant bacteria, especially biofilm-forming pathogens [12]. One of the strategies for obtaining electrospun materials with antimicrobial properties is the addition of essential oils (EO) and herbal extracts into polymeric nanofibers[4,9]. NFs can be produced at room temperature and can be very efficient for adding volatile antibacterial agents such as essential oils [3]. Herbal medicines are safe and have much fewer side effects than their synthetic counterparts [13]. Previously, various essential oils such as cinnamon, oregano, mint, lavender, eucalyptus, ginger, tea tree, and sage were used to obtain electrospun materials [9]. All of these natural additives have antimicrobial effects against a broad range of Gram-positive and Gram-negative bacteria. Moreover, they are easy to process, inexpensive, abundant and do not pose a risk of antimicrobial resistance [11].

Curcumin, a lipophilic polyphenol contained in the rhizome of Curcuma longa, has been used in traditional treatment, and these days is vastly used in food as a dietary spice. Due to the presence of conjugated double bonds in its chemical structure, this polyphenol acts as an effective electron donor to cancel the production of reactive oxygen species in many redox reactions [13]. Curcumin improves inflammatory conditions, metabolic syndrome, pain, and eye health by targeting cellular molecules [7].

Clove (Syzygium aromaticum L., Myrtaceae) is a fragrant plant that is extensively grown in tropical countries. It is abundant in volatile compounds and antioxidants like eugenol, β-caryophyllene, and α-humulene. Clove essential oil has been shown to have several biological activities that are beneficial to human health, such as antimicrobial, antioxidant, and insecticidal properties[14]. The main component of clove essential oil is eugenol, which is traditionally used as an analgesic and antiseptic in the prevention and treatment of tooth decay and periodontal disease [9]. Conventional methods such as cold pressing, hydro-distillation, and steam distillation are used to extract essential oils from plant feedstock [14].

In the present study, different antimicrobial agents were incorporated into electrospun PLA fibers to investigate the antibacterial effects of natural additions (Curcumin powder and Clove oil) on S. pyogenes and E. coli by reporting the capability of natural additions in inhibiting bacterial growth. In addition to studying the effect of viscosity of polymeric solutions, after adding each curcumin powder and clove's oil, on the fibers diameter of PLA.

Materials and Methods

Material:

Curcumin powder 99% (C12H20O6) was bought from Sigma Aldrich Chemical Co., USA. PolyLactic acid (PLA, average MW: 150 kDa, density 1.25 g/cm^3). PLA wire used in this research is commonly used in 3D printing, to recycle excess wire. Clove dried flowers were purchased from the local market. Dimethylformamide (DMF, density 0.95 g/cm^3) and Acetone (Ac, density 0.79 g/cm^3) were bought from POCH Sowinskiego, Poland.

An electrospinning device was used to create the nanofiber mats. The machine had a high voltage supply of 16 kV, a conical drum diameter of 15.8 cm, and a speed of 500 rpm. A 20 mL syringe and needles of 22 gauge were used. A trial-and-error method was used to determine the best spinning conditions, which were found to be approximately 12.2 kV, +22 kV, 0.35 kW, and a flow rate of 4 mL/h at an ambient condition of 40% RH and a temperature of 25°C. The fiber mats were collected on the surface of an aluminum foil. Several fiber mats were produced using a mixture of PLA and natural materials extract solution. They were then cleared in Table 1.

Clove oil extraction:

Clove oil was extracted by hydrodistillation method using Clevenger Apparatus. The specific weight of

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raw material was boiling for 90 minutes. After that, the oil was connected to the water supply from the chiller. Then, oils are collected and stored for later use.

Table 1. Naming the samples according to the ratio of the addition natural materials (Curcumin or Clove oil)

Sample	10% PLA	10% PLA+ 1% Natural Material	10% PLA+ 3% Natural Material	10% PLA+ 5% Natural Material	10% PLA+ 7% Natural Material	10% PLA+ 10% Natural Material
Curcumin	0	1	2	3	4	5
Clove oil	0	6	7	8	9	10

Polylactic acid solution preparation:
PLA wire was dissolved in 40/60% acetone/DMF by a hot plate-magnetic stirrer at 80°C to assemble a 10% PLA solution (by mass).

Preparation of electrospinning solutions:
Prepared clove oil, in addition to curcumin powder, and the PLA solution were blended at different ratios to prepare final solutions for electrospinning. Then, the solution was stirred for around 1 h at an elevated 70°C temperature to make a homogenous mix.

Characterization Techniques:
Three sets of experiments were examined. The first set of experiments involved measuring the viscosity of prepared solutions using a capillary viscometer known as the Ubbelohde. The Ubbelohde measures the time taken for a liquid to flow through a capillary of a specific volume. Using this data, the viscosity (v) of the liquid can be calculated using the formula shown in Eq. 1, where k is the constant of the viscometer, and t is the flow time of the liquid [15].

$$V = k * t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots. \ 1$$

If you're looking for nanofibers with specific diameters and morphologies, then you need to pay attention to the viscosity of the polymer solutions. By using viscous polymer solutions, you can achieve larger and more uniform nanofibers. Don't settle for less than the best. Choose the right viscosity and get the results you need.
Viscosity primarily determines the morphology of the fibers. Thick polymer solutions lead to uniform and bigger fiber [16,17].
In the second set, fabricated fiber mat samples were subjected to investigate the antibacterial efficacy by diffusion method against S. pyogenes and E. coli bacteria. The antimicrobial properties of PLA-fabricated fiber mats were determined using the agar disc diffusion method.
The experiment was initiated by transferring bacterial isolates (E. coli and S. pyogenes) from frozen glycerol stock solutions to plates. Following this, the plates were incubated for 24 hours at 37°C under aerobic conditions. The reason for this step was to facilitate bacterial growth. After incubation, a single colony was transferred to 5 mL of LB broth medium and incubated for another 24 hours at 37°C. This step was taken to allow the bacterial culture to grow.
A bacterial suspension was prepared and used to inoculate Mueller-Hinton agar plates. After drying for 5 minutes, sterile mats were placed on the plates. The plates were incubated for 24 hours at 37°C under aerobic conditions. Finally, the zone of inhibition surrounding each sample was measured [11].
In the third set, structural analysis was performed of the resulting mats. The morphology of the prepared nanofibres was examined using a scanning electron microscope (SEM), and their diameter was determined through ImageJ software.

Results and Discussion

Viscosity of Prepared Solutions:
The viscosity of the polymer solutions can be controlled by varying the concentration, and it is the most important factor that influences the fiber morphology and diameter values [15]. The viscosity of all prepared polymeric solutions was measured before electrospinning. The results are in Table 2.

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Table 2. Viscosity of prepared polymeric solutions (mPa.s) .

Sample No.	Viscosity	Sample No.	Viscosity
0	110.2	-	-
1	112.3	**6**	110.5
2	114.8	**7**	111.7
3	116.5	**8**	112.8
4	117.9	**9**	113.2
5	120.9	**10**	113.9

The viscosity of the polymeric solutions increases with a higher concentration of the curcumin powder and oil clove. The viscosity of the polylactic acid solution increased from (110.2 mPa.s) to (112.3 mPa.s) and (110.5 mPa.s) for the sake of adding only 1% of curcumin powder and oil cloves, respectively. Adding curcumin powder increased solution viscosity more than clove oil at the same concentration.

The increase in the viscosity of the polylactic acid solution when clove oil is added may be due to the formation of hydrogen bonds between the eugenol and the polymer. As the concentration of added oil increases, the number of hydrogen bonds formed increases, resulting in increased viscosity [18].

It is also noted that the viscosity of the polylactic acid solution when adding curcumin powder is higher than the viscosity of the solution with clove oil for the same concentrations. This may be because curcumin has more functional groups capable of forming hydrogen bonds (hydroxyl and carbonyl groups), giving a higher viscosity [19].

Antibacterial activity:

The antibacterial activity test of the electrospun mats samples was carried out against two types of bacteria: gram-positive (S. pyogenes) and gram-negative (E.coli) bacteria. The zones of inhibition for all specimens are shown in Table 3.

Table 3. Antimicrobial activities of PLA, curcumin/PLA and Clove oil /PLA electrospun mats against S. pyogenes and E. coli.

Sample No.	Zone of inhibition (mm)		Sample No.	Zone of inhibition (mm)	
	E.coli (-)	S. pyogenes **(+)**		E.coli (-)	S. pyogenes **(+)**
0	0	0	**-**	-	-
1	1	2	**6**	1	2
2	2	2.5	**7**	2	2
3	2.5	3	**8**	4	4.5
4	3	3.5	**9**	5	5
5	4	3.5	**10**	6	6.5

The PLA sample did not show any antibacterial efficacy. The samples containing curcumin showed antibacterial activity against the two types of bacteria, and it was observed that this activity increased with the increase of the concentration of curcumin, and the antibacterial activity was greater against gram-positive bacteria than with gram-negative bacteria at the same concentrations of curcumin. Also, samples containing clove oil showed antibacterial activity against the two types of bacteria, and with an increase in the concentration of clove oil in the samples, the area of inhibition increased. Upon comparing the effectiveness of mats containing curcumin powder and clove oil, it was observed that clove oil exhibited the most effective antibacterial properties in the samples. This can be attributed to the essential oil's lipophilic properties, which enable it to easily permeate through the cell wall and membrane, leading to the breakdown of the cell membrane and ultimately resulting in cell death. The interaction of the essential oil components with sugars, fatty acids, and phospholipids also plays a significant role in this process [20].

Structural analysis:

An electron microscopy test was performed for the resulting samples, where the average fiber diameters of the PLA sample were 198 nm, as displayed in Fig. 1.

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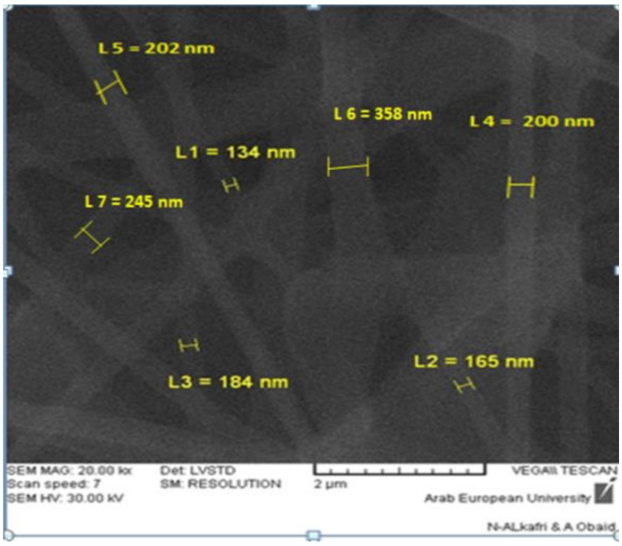

Figure 1. PLA (10%) Electrospun Nanofibers

Samples 1, 2, 3, and 4 containing curcumin in addition to PLA, in Fig. 2. It was noticed that the average diameter of the fibers ranged between 676-961 nm.

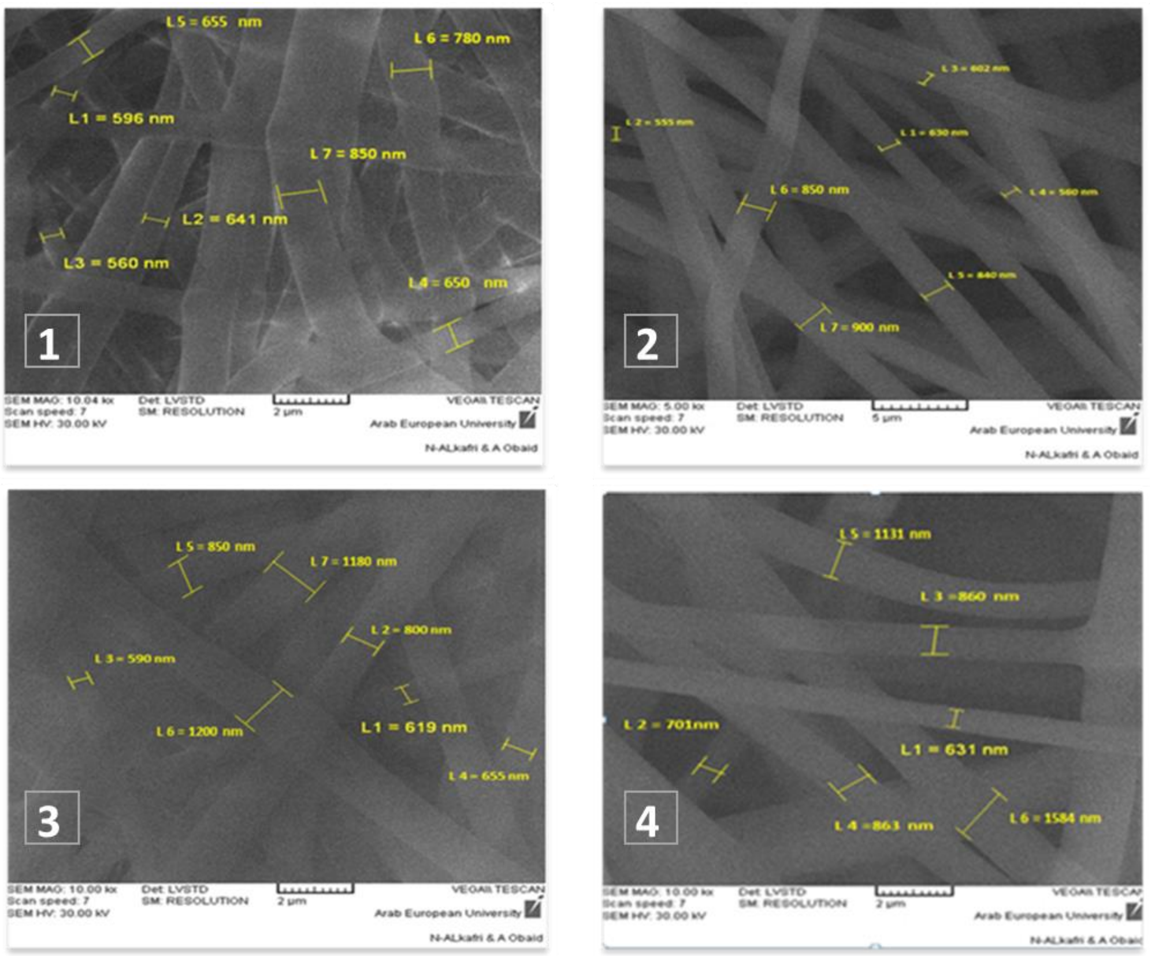

Figure 2. PLA (10%) + Curcumin Electrospun Nanofibers

There is an increase in the average fiber diameters with the increase in the concentration of curcumin, and this can be explained by the increase in the viscosity of the solutions, as the increase in

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the diameters is directly proportional to the increase in the viscosity of the polymeric solution. This is consistent with what was reported in the literature [21]. In Fig. 3, it is shown that samples 6, 7, 8, and 9 containing clove oil had an average fiber diameter ranging between 339-and 608nm. An increase in the concentration of clove oil led to an increase in the average fiber diameter due to the increase in solution viscosity. However, the samples composed of polylactic acid and curcumin had greater diameters than those composed of polylactic acid and clove oil. This is because the viscosity of polylactic acid solutions with curcumin is greater than that of polylactic acid solutions with clove oil, in addition to the volatilization of part of the clove oil during preparation and heating of the solution.

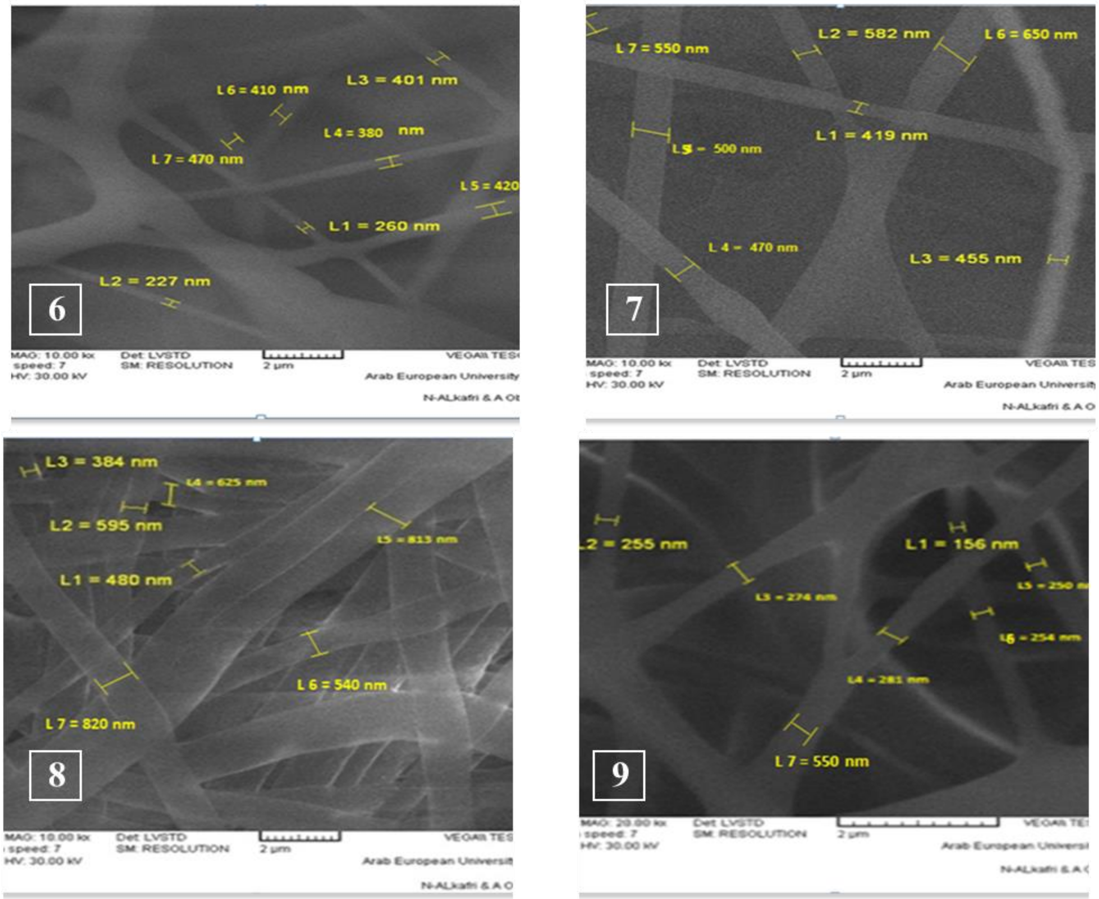

Figure 3. PLA (10%) + Clove Oil Electrospun Nanofibers

Table 4 shows the average diameters of fibers in the fabricated mats of the samples, for samples containing curcumin, the average fiber diameters ranged between 676-961 nm. In comparison, samples containing clove oil had an average diameter of fibers between 339-608nm, As compared to PLA-clove oil solutions, all PLA-curcumin solutions exhibit a higher viscosity.

Table 4. Diameters of fibers in the fabricated mats of the samples

Sample No.	diameters of fibers (nm)	Stander deviation SD	Difference Factor (%)
0	198	43.22	0.21
1	676	102.7	0.15
2	706	149.5	0.21
3	842	255.7	0.30
4	961	350.2	0.36
6	339	118.6	0.34
7	366	89.10	0.24
8	518	80.56	0.15
9	608	162.5	0.26

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Conclusion

In the present study, the electrospinning technique was used in the manufacture of mats consisting of polylactic acid polymer (PLA) in addition to curcumin and clove oil (as natural antibacterial materials).
The samples were then prepared using a locally manufactured conical compound electro-spinner. Using a scanning electron microscope, it was found that the average diameter of the fibers of the polylactic acid sample is 177 nm. It is noted that the diameters of the fibers produced using polymer and natural materials have larger diameters, especially when curcumin powder is added. The activity of the produced samples against bacteria resistance against two types of Gram-negative and Gram-positive bacteria was tested. It was found that samples containing natural materials showed antibacterial activity against both types of bacteria. Increasing the concentration of the additive resulted in an increase in the diameter of the inhibition zone. This intends to make it suitable for medical and other applications such as masks, bandages, and tissue engineering. But all of this requires more experiments and tests, which we hope to do in the future.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Al-Baath University

Authors' Contribution Statement

A.B. conducted experiments, analyzed and interpreted received data. G.T. and H.B. conceived of the presented idea, developed the theory, and contributed to the design and implementation of the research. H.S. wrote, translated and proofread the manuscript into English. All authors discussed the results and contributed to the final manuscript.

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تأثير مسحوق الكركم وزيت القرنفل على خصائص الألياف المنتجة بتقنية الغزل الكهربائي

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الخلاصة

الغزل الكهربائي هو طريقة بسيطة للحصول على ألياف نانوية. والتي تمتلك خصائص مميزة مثل المساحة الكبيرة بالنسبة للوزن والمسامية العالية، مما يجعلها جذابة للعديد من التطبيقات. في هذا البحث استخدمت تقنية الغزل الكهربائي للحصول على شبكات لا منسوجة من الألياف النانوية باستخدام بولي لاكتيك أسيد (PLA) مع إضافة مواد هي مسحوق الكركم وزيت القرنفل كمواد طبيعية مضادة للبكتيريا. لتحضير الأغشية، حضر محلول البولي لاكتيك أسيد بتركيز 10% في مزيج من الأسيتون وثنائي ميتيل فورم أميدز أضيفت المواد الطبيعية إلى محلول البوليمير بعدة تراكيز 1، 3، 5، 7 و10%. وقيست لزوجة هذه المحاليل. ثم حضرت العينات باستخدام جهاز غزل كهربائي ذو مجمع مخروطي مصنع محلياً. باستخدام المجهر الإلكتروني الماسح، تبين أن متوسط أقطار الألياف النانوية لعينة البولي لاكتيك أسيد هي 177 نانومتر. ويلاحظ أن أقطار الألياف النانوية المنتجة باستخدام البوليمير والمواد الطبيعية، تمتلك أقطاراً أكبر وخاصة عند إضافة الكركم، حيث تراوحت أقطار الألياف بين 485 و764 نانومتر. اختبرت فعالية العينات المنتجة في مقاومة البكتيريا اتجاه نوعين من البكتيريا سالبة وموجبة غرام. تبين أن العينة المكونة من البوليمير فقط لا تمتلك أي مقاومة للبكتيريا. بينما تظهر العينات الحاوية على المواد الطبيعية فعالية مضادة للبكتيريا ضد نوعي البكتيريا. ولوحظ ازدياد هذه الفعالية بازدياد تركيز المادة المضافة مما أدى إلى ارتفاع قطر منطقة التثبيط. مما يجعلها مناسبة للتطبيقات الطبية وغيرها.

الكلمات المفتاحية: مقاومة البكتيريا، زيت القرنفل، الكركم، الغزل الكهربائي، الألياف النانوية، بولي لاكتيك أسيد.