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Antibacterial characterization of ciprofloxacin-doped electrospun of low molecular weight polyethylene oxide (PEO) and sodium alginate (NaAlg) nanofibers

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Crosslinking technique;
Ciprofloxacin

Abstract

Producing nanofibers using the electrospinning technique is a developed method that is widely used and of significant interest nowadays. This technique can be applied using various types of polymers. This research aimed to investigate the antibacterial PEO-NaAlg nanofiber fabrication. The fiber fabrication was examined under various viscosities of electrospinning solution. The electrospun nanofiber fabrication focuses on blending polyethylene oxide (PEO) with a molecular weight of 200-300 kDa, mixed with sodium alginate (NaAlg) of three different viscosities: 150 cP, 300 cP, and 730 cP to study how the viscosity of the solution affects the morphology of electrospun nanofibers. The PEO-NaAlg electrospun nanofiber was enhanced for water insolubility by crosslinking with calcium chloride ($CaCl_2$). The additional antibacterial property of the nanofiber by loading an antibacterial agent potentially against the growth of bacteria, was investigated. Antibacterial drug, ciprofloxacin at varying amounts of 0.05%w/v, 0.20%w/v, and up to 0.25%w/v was loaded to PEO-NaAlg solution and conducted electrospinning. The effectiveness of the antibacterial electrospun nanofiber was evaluated by testing its ability to inhibit the growth of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The inhibition area before and after crosslinking was observed. The results showed that the acquired nanofiber formation required 7%w/v of 200 kDa to 300 kDa of PEO, and blending 1%w/v NaAlg of 150 cP can certainly retain fiber morphology after crosslinking. Moreover, nanofibers loaded with ciprofloxacin effectively inhibit the growth of *E. coli*.

1. Introduction

Electrospinning is a method for fabricating fibers from polymer solution, producing fibers with diameters ranging from nanometers to micrometers [1]. This technique requires three main components, including a high-voltage power supply, a syringe pump, and a metallic collector plate or rotary collector. High voltage is applied to the needle tip, creating an electric charge that moves from the polymer solution toward the metallic collector. This process stretches the droplet of polymer solution into the fiber formed by inducing the electrical charges [2]. The fabricated nanofibers have nanoscales, which are an invisible size to the human eyes. Electrospun nanofibers are often referred to as nanofibers when the diameter of the fiber is thinner than roughly 500 nm [3]. Various parameters involved in the electrospinning process affect the morphology and diameter of the fiber. These parameters are classified into process parameters, including applied voltage, flow rate, and distance between the needle and metallic collector, solution parameters including concentration, viscosity, conductivity, molecular weight, etc., and environmental parameters including temperature and humidity [4]. The small size of nanofibers offers numerous advantages applied in various applications, with biomedical applications being prominent. Research in biomedical applications has extensively explored the development potential of electrospinning techniques [5]. This technique can fabricate fibers from natural, synthetic, and co-polymers, making them biodegradable and biocompatible [6]. This characteristic enables their use in biomedical applications, including wound dressing fabrication and scaffolds in tissue engineering applications [7–13]. Polyethylene oxide (PEO) and sodium alginate (NaAlg) were utilized to fabricate the nanofibers using the electrospinning technique [14–16]. PEO, being a polymer of ethylene oxide, possesses biocompatible, biodegradable, and non-toxic, which are suitable for various biomedical applications [17]. Given its polymer nature, PEO alone can be employed to create electro-spun nanofibers through the electrospinning technique, but the stability of PEO fiber is low, and water-soluble. Therefore, modified fiber construction is required to improve the properties of electrospun nano-fibers. Sodium alginate is a natural polysaccharide produced from brown algae, obtained from the sodium salt of alginic acid [18]. NaAlg, in isolation, lacks the capacity to form electrospun nanofibers. However, when NaAlg is blended with PEO, the hydroxyl groups (–OH) of NaAlg interact with the hydrogen bond of the ether oxygen (–O–) in the PEO structure [19]. This reaction enhances the chain and flexibility of the resulting electrospun nanofibers of PEO-NaAlg [20]. NaAlg is an ionic salt. When combined with PEO, it increases the ion charge of the solution, thereby increasing the conductivity and surface tension of the electrospinning process [21]. Consequently, this enhancement leads to an improvement in the fabrication of fiber, yielding electrospun nanofibers without bead particles. The application of fiber requires the properties of insolubility, however, PEO and NaAlg are water-soluble even if they are blended. To improve fiber insolubility, the

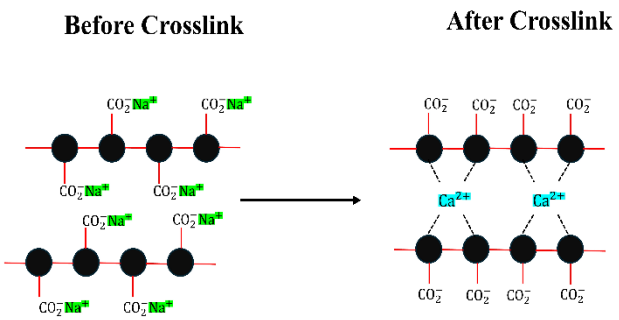

Figure 1. The structure of the PEO-NaAlg before crosslinking and the PEO-Alg after crosslinking.

inter-chain association potentially achieves many target properties of fiber [22,23]. The most commonly used crosslinking agent with NaAlg is calcium chloride ($CaCl_2$) [23]. In the interactions, the Ca^{2+} ion from calcium chloride replaces the Na^+ ion and bonds with two carboxylate groups of alginate molecules, resulting in the formation of a three-dimensional network structure, as shown in Figure 1 [24]. This leads to enhanced water insolubility of the electrospun nanofibers.

Several researchers have successfully fabricated nanofibers from PEO-NaAlg using PEO with a molecular weight above 600 kDa, mixed with NaAlg [14–17] Additionally, there are also researches reports that low molecular weight of PEO (lower than 600 kDa) blended with NaAlg cannot form the electrospun nanofibers without bead particles due to insufficient chain entanglement to form a continuous fiber [14,16,25]. However, the lower molecular weight of PEO results in lower solution viscosity, making it more readily soluble in water and easier to form a homogeneous solution, leading to a more straightforward process than higher molecular weight PEO [26]. Moreover, low molecular weight PEO solubility allows additional agents to mix homogeneously without causing excessive viscosity, thereby reducing the need for surfactants to lower solution viscosity for successful electrospun nanofiber formation. Therefore, low molecular weight PEO is an attractive and challenging material for investigating the antibacterial electrospun nanofibers, which can be further applied in biomedical applications. Electrospun nanofibers from PEO-NaAlg have been applied in various biomedical applications, including wound dressing and scaffolding in tissue engineering. An additional antibacterial property could be essential because it can help reduce contamination, which causes infection and is harmful to cell seeding for tissue engineering [27]. Adding antibacterial agents is one of many factors that play an important role in the fabrication of electrospun nanofibers for medical uses. Various antibacterial agents and antibiotic drugs affecting electrospun nanofiber formation have been investigated, including anthocyanin [28], curcumin [29], centella asiatica extract [30], amoxicillin [31], penicillin [32], ciprofloxacin [14,33], etc. These agents can be added to the solution for fiber fabrication, which helps to prevent the growth of bacteria, reduce inflammation from the wound, and promote the recovery of damaged tissue in the case of antibacterial wound dressing application [34,35].

Ciprofloxacin is an antibiotic drug in the fluoroquinolone class that has been effective against both gram-positive and gram-negative bacteria. Ciprofloxacin inhibits DNA gyrase, which is known as topoisomerase II and topoisomerase IV, thereby stopping DNA activities such as replication and transcription processes [36]. It has been used to treat millions of patients, both adults and children, for various conditions, including skin infections and urinary tract [36]. Therefore, it is considered for biomedical applications. To investigate the efficiency of antibiotic property in PEO-NaAlg electrospun nanofiber, this research focused on varying the amount of ciprofloxacin mixed with PEO-NaAlg to observe the inhibition of bacteria.

From the literature review, the previous studies have explored the effects of low molecular weight PEO combined with NaAlg influences the formation of electrospun nanofibers loaded with the antibacterial agent, but the findings remain insufficiently detailed. Therefore, this research aims to investigate the fabrication of the antibacterial electrospun nanofibers based on low molecular weight 200 kDa to 300 kDa PEO-NaAlg by the electrospinning technique. The insolubility of the nanofiber is enhanced through a crosslinking method with calcium chloride. The effect of PEO concentration and NaAlg viscosities is examined to obtain an appropriate condition for nanofiber fabrication. Additionally, the concentrations of the antibacterial agent ciprofloxacin loaded in PEO-NaAlg nanofibers are examined to observe their effect on bacterial growth.

2. Materials and methods

2.1 The set-up of electrospinning device

The electrospinning device in this research consists of 3 main parts, including a high-voltage power supply, a syringe pump that contains the syringe with a blunt tip, and a metallic collector plate covered with aluminum foil. The electrospinning process was carried out with 15 kV of applied voltage, 0.5 $mL{\cdot}h^{-1}$ of flow rate, and 15 cm distance between the needle and collector plate. The electrospinning device is shown in Figure 2.

2.2 Electrospinning solution for fiber fabrication

Polyethylene Oxide (PEO) (POLYOX™ WSR N750, DuPont) with a molecular weight ranging from 200 kDa to 300 kDa and three different viscosities of sodium alginate (NaAlg), including 150 cP (TCS mart), 300 cP (Krungthepchemi), and 730 cP (Chemrich) were used to examine the fabrication of electrospun nanofibers. The solution was prepared by mixing PEO at 4%w/v to 7%w/v and NaAlg of 1%w/v to 2%w/v dissolved in deionized water by stirring until it was homogeneous at room temperature for 24 h. The viscosity of the homogeneous solutions was measured using a rotational viscometer (NDJ-4). The measurement of viscosity was performed at room temperature, 25°C. The rotor (ranging from 0 to 4) and speed (rpm) were selected based on the viscosity of the solution. The NDJ-4 viscometer offers eight selectable speeds: 0.3 rpm, 0.6 rpm, 1.5 rpm, 3 rpm, 6 rpm, 12 rpm, 30 rpm, and 60 rpm. Less viscous solutions were measured using higher speeds and lower rotor numbers. The rotor and speed were adjusted according to the instrument's indicated range; settings were chosen when the pointer stopped within the appropriate measuring range of each rotor-speed combination. The viscosity (in centipoise, cP) was calculated by multiplying the value at which the pointer stopped (a) by the corresponding coefficient (k) depending on the rotor and the speed used, based on the formula: η (real viscosity) = a x k. Each measurement was repeated three times to ensure accuracy.

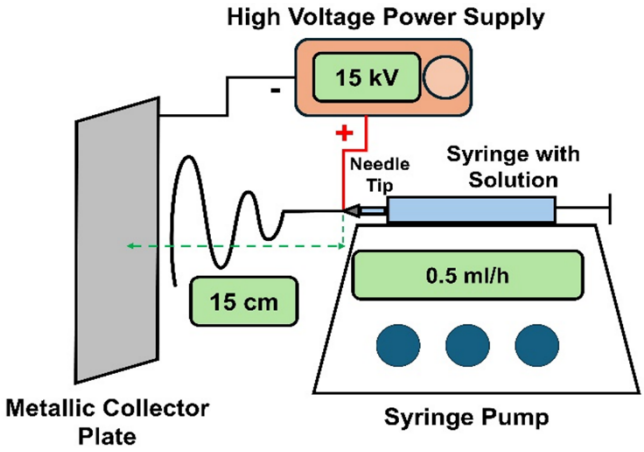

Figure 2. The setup of the electrospinning device.

2.3 Crosslink method

The crosslinking process was performed following the published research [14]. The electrospun nanofiber was submerged in 70% ethanol for 1 min to facilitate further crosslinking reaction, enhancing the stabilization process [14,37]. After that, the crosslinking process was performed by submerging electrospun samples in 2 wt% $CaCl_2$ in a volume ratio of 1:5 (ethanol : water) for 15 min. To ensure the water insolubility of electrospun nanofibers, the samples were tested by submerging in deionized water for 1 min.

2.4 FTIR analysis

The spectrum of the electrospun nanofibers was used to identify the composition of the fiber by the Attenuated Total Reflectance (ATR) from the FTIR spectrometer (IRPrestige-21, Shimadzu). Transmittance data were measured at wavenumber ranging from 4000 cm^{-1} to 400 cm^{-1}.

2.5 SEM analysis

To characterize the morphology of nanofibers, electrospun samples were coated with gold (Q150R Plus, Quorum Technologies Ltd) and observed using a scanning electron microscope (SEM) (Apreo S, ThermoFisher Scientific). The SEM images were taken at various magnifications, ranging from 8000x to 40000x. The accelerated voltage is 15 kV. The diameter of the electrospun nanofiber was analyzed by using ImageJ software version 1.49k [38]. The diameter of the fibers was measured on SEM images by using the ImageJ version 1.49 program.

2.6 DSC analysis

Differential scanning calorimetry (DSC; DSC 3500, NETZSCH) was performed to investigate the thermal properties of pure PEO and electrospun nanofibers before and after crosslinking. The electrospun nanofibers were cut into approximately 5 mm pieces, and about 5 mg to 10 mg of each sample was sealed in aluminum DSC pans and analyzed under a nitrogen atmosphere. The samples were heated from 25°C to 95°C at a heating rate of 20°C·min^{-1} to observe the thermal properties of PEO and to confirm that PEO was removed after crosslinking [39]. An empty aluminum pan was used as a reference. The DSC thermograms were recorded to identify the melting behavior of PEO and evaluate changes in thermal transitions.

2.7 Drug loading

Ciprofloxacin is an antibiotic drug that was used in this research to evaluate the antibacterial activity of PEO-NaAlg electrospun nanofibers. Ciprofloxacin hydrochloride (MySkinRecipes®) at concentrations of 0.05%w/v, 0.2%w/v, and 0.25%w/v was added to the PEO-NaAlg solution. The solution was stirred until it became homogeneous. These drug-loaded PEO-NaAlg solutions were electrospun using the same electrospinning parameters as PEO-NaAlg without drug loading.

2.8 Preparation of antibacterial testing

Escherichia coli (*E. coli*) *TISTR 527* and *Staphylococcus aureus* (*S. aureus*) *TISTR 746* were used to test the antibacterial property of electrospun nanofiber. These bacteria strains were obtained from Thailand Institute of Scientific Technological Research (TISTR). Bacteria were cultivated in rich nutrient broth (Himedia) of 0.5%w/v peptone, 0.5%w/v NaCl, 0.15%w/v yeast extract, and 0.15%w/v beef extract at 37°C with shaking at 150 rpm for 24 h. After incubation, the bacterial cultures were serially diluted to 10^{-3} and spread onto a rich nutrient agar medium with 100 µL. The electrospun samples were cut precisely into discs with 6 mm diameter and placed on the spread plates and incubated at 37°C for 24 h. To obtain the validated results, the testing experiment was repeated with three electrospun samples for each condition. The diameter of the inhibition zone was measured using the ImageJ version 1.49 program to evaluate the antibacterial properties of the electrospun samples.

3. Results and discussion

3.1 Electrospun fiber fabrication

PEO is the core polymer material used for nanofiber fabrication by the electrospinning technique. The effect of PEO concentrations at 200 kDa to 300 kDa on the formation of nanofibers was observed. The concentration of PEO varied from 4%w/v to 7%w/v. The concentration of PEO affects the average diameter of the fiber and its morphology, as illustrated in Table 1 and Figure 3, respectively. Increasing the concentration of PEO results in an increase in solution viscosity and average diameter of nanofiber. Among four concentrations of PEO, the smallest diameter was observed at 32.34 nm by 4%w/v PEO, and the highest diameter was 75.23 nm by 7%w/v PEO. The SEM results with 8000x magnification reveal the bead particles that appeared on 4%w/v to 6%w/v PEO, as shown in Figure 3. However, at the higher PEO concentration of 6%w/v, fiber became more apparent. The completed fiber formation was observed on 7%w/v PEO. These results indicated that the increased concentration of PEO led to the disappearance of bead particles and achieved fiber formation. Therefore, 7%w/v is the minimum concentration of PEO at 200 kDa to 300 kDa, enabling fiber fabrication without undesired bead particles, and it was used to study with NaAlg to improve fiber stabilization.

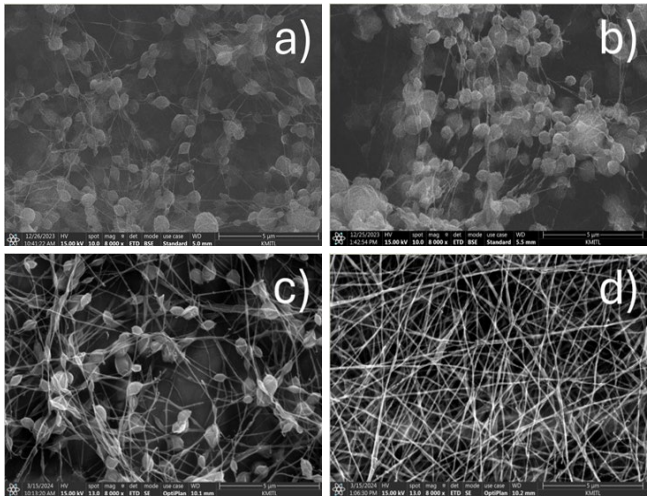

Figure 3. SEM images 8000x of the morphology of electrospun nanofibers with different PEO concentrations: (a) 4%w/v, (b) 5%w/v, (c) 6%w/v, and (d) 7%w/v.

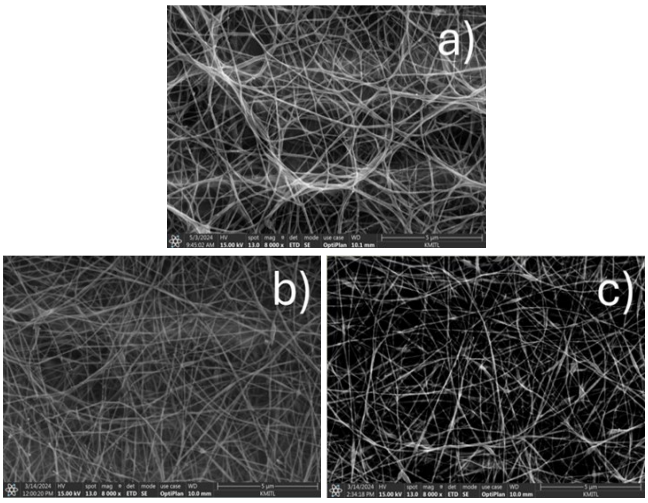

Figure 4. SEM images 8000x of the electrospun nanofiber from 7%w/v PEO mixed with 1%w/v NaAlg of (a) 150 cP, (b) 300 cP, and (c) 730 cP.

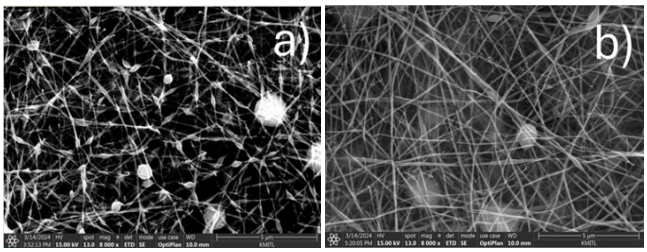

Figure 5. SEM images 8000x of the electrospun nanofiber from 7% w/v PEO mixed with 2%w/v NaAlg of a) 150 cP, and b) 300 cP viscosity.

Because viscosity is an important parameter that can affect the morphology of the electrospun nanofiber [38]. The suitable range of viscosity of PEO solution for completing fiber formation is between 100 cP and 2000 cP [40]. Adding NaAlg into the 7%w/v PEO solution increases the viscosity of the solution. Therefore, various solution viscosities based on 7%w/v PEO at 150 cP, 300 cP, and 730 cP of NaAlg were examined. The amount of NaAlg at 1%w/v and 2%w/v was added, and the final viscosity of the solutions was measured, as presented in Table 2. The morphology of the nanofiber observed by SEM at 8000x magnification is illustrated in Figure 4-5 of 1%w/v and 2%w/v of NaAlg, respectively. The completed fiber formation was certainly achieved at 745 cP to 1,315 cP by adding 1%w/v of NaAlg, as shown in Figure 4. However, an increase in the solution viscosity affects the surface tension of the nanofiber and leads to the formation of bead particles, as shown in Figure 5 when 2%w/v of NaAlg was added. At the highest viscosity of 12,800 cP, the solution became extremely thick and solidified easily due to the high guluronic acid content in NaAlg, which prevented the solution from flowing out of the needle [41]. Moreover, NaAlg is an ionic salt, and the high amount of NaAlg impacts the over-conductivity of the solution. This causes an unstable fiber formation [42]. Based on these results, 7% of PEO mixed with 1%w/v NaAlg of three viscosities, 150 cP, 300 cP, and 730 cP could achieve nanofiber formation and were selected for further improvement of insolubility through the crosslinking process.

Table 1. The solution viscosity and the average diameter of 4%w/v to 7%w/v PEO concentration at 200 kDa to 300 kDa.

Concentration of PEO [%w/v]	Viscosity [cP]	Average diameter [nm]
4	39.5	32.34
5	44	37.40
6	100	55.13
7	225	75.23

Table 2. The solution viscosity for electrospun fibers under the different concentrations and viscosities of NaAlg.

NaAlg viscosity [cP]	NaAlg concentration [%w/v]	PEO concentration [%w/v]	Final viscosity of PEO mixed with NaAlg [cP]
150	1	7	745
	2	7	1,450
300	1	7	795
	2	7	2,760
730	1	7	1,310
	2	7	12,800

3.2 The crosslinked fiber

Water insolubility of fiber can be improved by crosslinking between polymer chains, in which PEO-Na^+ alginate fiber is turned into Ca^{2+} alginate, forming the inter-chain crosslink network in fiber [37]. The electrospun nanofibers from 7%w/v PEO mixed with 1%w/v NaAlg of different viscosities were electrospun for 8 h. The nanofiber morphology and average diameters before and after the crosslinking process were observed. Figure 6 displays the SEM results at 40000x magnification, showing that the fibers retain their morphology after crosslinking. The average diameter of fibers was measured and calculated as shown in Figure 7. Before crosslinking, the average diameter of PEO-NaAlg electrospun fiber at viscosities of 745 cP, 795 cP, and 1,310 cP was 72.34 nm, 80.84 nm, and 89.37 nm, respectively. These results indicate that the average diameter of the fiber before crosslinking increases with increasing solution viscosity [43]. However, the average diameter after crosslinking was reduced compared to the average size before crosslinking, as shown in Figure 7. The average diameter of the fiber after crosslinking at viscosities of 745 cP, 795 cP, and 1,310 cP was 52.88 nm, 51.82 nm, and 48.85 nm, respectively. This is due to the removal of PEO from the fibers, as it is soluble in both ethanol and water, resulting in fiber shrinkage after crosslinking. This observation could be proved by FTIR and DSC results. However, removing PEO did not affect the fiber structure [37,44].

FTIR analysis is used to confirm the success of the crosslink process. Before crosslinking, the fiber consists of 7%w/v PEO and 1%w/v NaAlg. PEO and NaAlg can fabricate certain nanofibers as they interact together between the hydroxyl group (–OH) of NaAlg and the hydrogen bond of the ether group (–O–) in the PEO, which was confirmed by the stretching at 3200 cm^{-1} to 3600 cm^{-1} of the hydroxyl group of NaAlg compared to the NaAlg spectrum as shown in Figure 8. After blending, the ether spectrum of PEO was shifted from 1100 cm^{-1} to 1095 cm^{-1} After blending, the ether spectrum of PEO was shifted from 1100 cm^{-1}, and the spectrum of the carboxylate group (COO^-) in the NaAlg was shifted from 1593 cm^{-1}. After blending, the ether spectrum of PEO was shifted from 1100 cm^{-1} to 1612 cm^{-1} After blending, the ether spectrum of PEO was shifted from 1100 cm^{-1} [15]. After crosslinking, PEO was removed due to its solubility corresponding to the peak of ether from PEO disappeared. Because there was an exchange from Na^+ to Ca^{2+} ions in the alginate molecules, the carboxylate group peaked at a higher frequency of 1612 cm^{-1} due to the ion exchange interaction compared to before crosslinking [45]. The spectrum at 3200 cm^{-1} to 3600 cm^{-1} indicates the hydroxyl group contained in NaAlg. The crosslinking process decreased the hydrogen bonding between the hydroxyl group (–OH) of NaAlg and the ether group (–O–) due to the exchange from Na^+ to Ca^{2+} ions, resulting in the formation of a network structure that enhances the stability of the electrospun and improves fiber insolubility property [46]. DSC was performed to characterize the thermal properties of PEO, confirming that the PEO was removed after crosslinking. As shown in Figure 9, the DSC thermogram of pure PEO (7%w/v) and the electrospun nanofibers before crosslinking exhibited a melting peak in the range of 60°C to 70°C, which corresponds to the melting temperature of PEO [47]. Pure PEO exhibited a melting peak at 63.7°C, while the electrospun nanofibers before crosslinking showed a melting peak at 65.4°C. In contrast, no melting peak was observed in this temperature range for the electrospun nanofibers after crosslinking, confirming that PEO was removed during the crosslinking process.

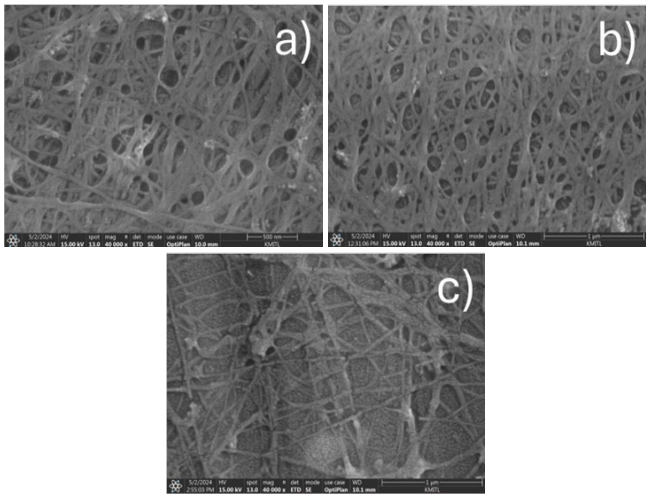

Figure 6. SEM images 40000x of fiber morphology after crosslinking. The solution of 7%w/v PEO mixed with 1%w/v NaAlg of (a) 150 cP, (b) 300 cP, and (c) 730 cP.

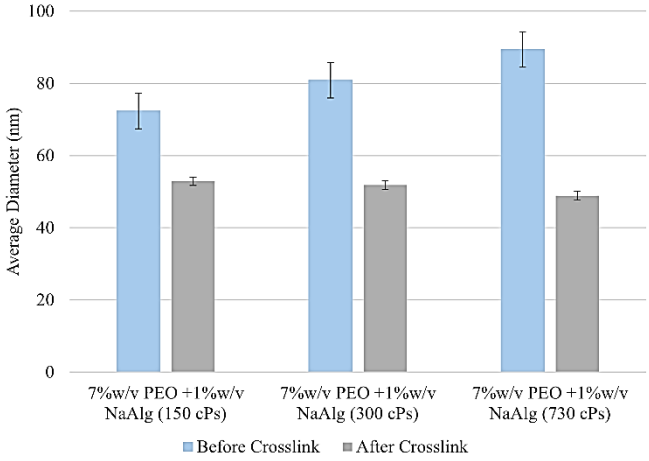

Figure 7. The average diameter of fiber fabricated by 7%w/v of PEO mixed with 1%w/v NaAlg of different viscosities before crosslinking (blue) and after crosslinking (grey).

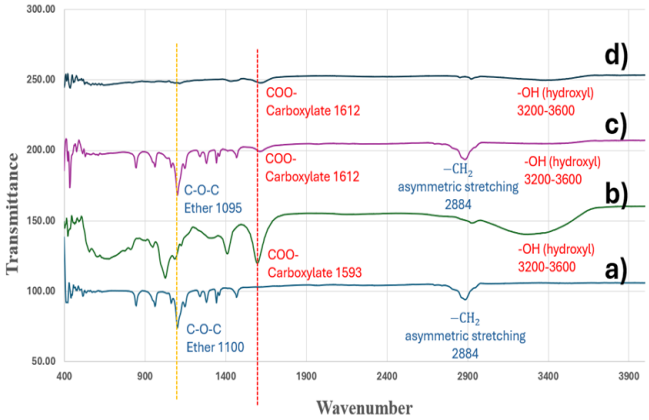

Figure 8. FTIR spectra of (a) 7%w/v PEO, (b) NaAlg (150 cP), 7%w/v PEO mixed with 1%w/v NaAlg (150 cP) of (c) before crosslinking, and (d) after crosslinking.

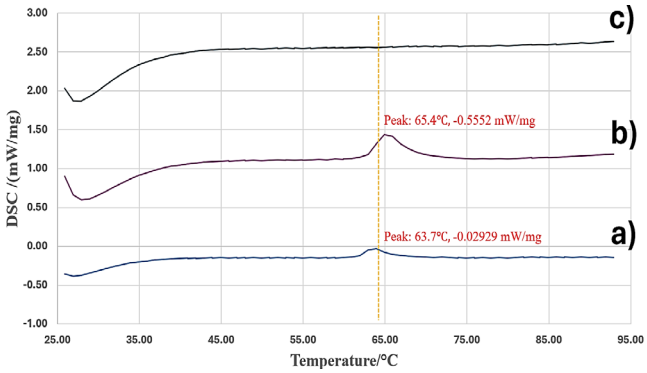

Figure 9. DSC plot of (a) 7%w/v PEO, 7%w/v PEO mixed with 1%w/v NaAlg (150 cP) of (b) before crosslinking, and (c) after crosslinking.

Based on the structural retention of the fiber after the crosslinking process, a composition of 7%w/v of PEO with 1%w/v of 150 cP NaAlg could certainly retain the structure and the average fiber size. Therefore, this condition was selected to investigate the additional antibacterial property of the fiber.

3.3 Antibacterial nanofiber

Since *E. coli* and *S. aureus* are used as indicators to assess hygiene conditions and sanitation, these two bacteria were selected to study the antibacterial property of fiber [48]. The additional antibacterial activity of electrospun nanofibers was tested by loading different amounts of ciprofloxacin to observe the inhibition zones of *E. coli* and *S. aureus*. The 7%w/v PEO with 1%w/v of 150 cP NaAlg solution was mixed with ciprofloxacin at concentrations of 0.05% up to 0.20%w/v, and then a homogeneous ciprofloxacin-containing polymer solution was electrospun for 8 h. The amount of ciprofloxacin was determined based on the study of the electrospun alginate nanofibers loaded with ciprofloxacin hydrochloride as reported by A. Kyziol [14]. Moreover, these concentrations of ciprofloxacin loading covered the range of effectiveness against the bacteria's growth in the nanofiber form, as discussed by T. Thairin [49]. The electrospun nanofibers of the PEO-NaAlg with 0.05%w/v ciprofloxacin solutions retain a smooth fiber before crosslinking, as shown in Figure 10(a) analyzed by SEM at 40000x magnification. However, a rough fiber was observed after crosslinking, as shown in Figure 10(b). When the ciprofloxacin was increased to 0.20%w/v, the smooth nanofibers were successfully retained, as shown in Figure 11, observed under SEM at 40000x magnification. Before crosslinking, the average fiber diameters of PEO-NaAlg loaded with 0.05%w/v and 0.20%w/v ciprofloxacin were 81.13 nm and 85.50 nm, corresponding to increases of 12.15% and 18.19%, respectively, when compared to the fiber without ciprofloxacin, which was 72.34 nm (Figure 4(a) and Figure 7). These results indicated that ciprofloxacin did not affect the formation of fiber, but the fiber diameter expanded and fused. Additionally, after crosslinking, the average diameter of the ciprofloxacin-loaded fibers increased compared with that before crosslinking, which can be attributed to the swelling of the nanofibers induced by ciprofloxacin during the crosslinking process [14,50]. Rough fiber surfaces were observed at a ciprofloxacin loading of 0.05%w/v. Despite these changes, the fibers retained their overall morphology and insolubility.

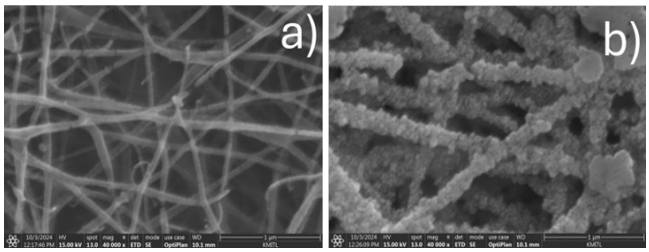

Figure 10. SEM images 40000x of PEO-NaAlg electrospun loaded with 0.05%w/v ciprofloxacin of (a) before crosslinking, and (b) after crosslinking.

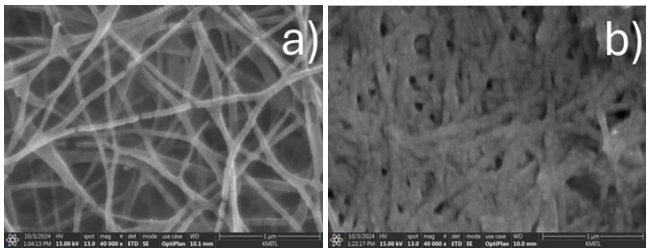

Figure 11. SEM images 40000x of PEO-NaAlg electrospun loaded with 0.20%w/v ciprofloxacin of (a) before crosslinking, and (b) after crosslinking.

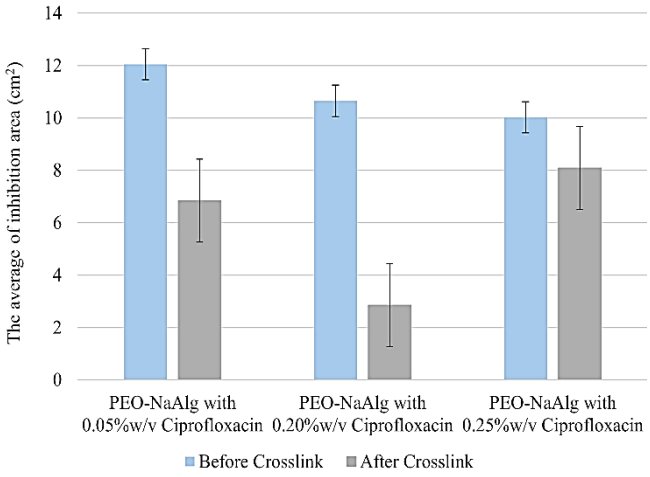

Figure 12. The average inhibition area of electrospun containing ciprofloxacin against *E. coli*, before crosslinking (blue) and after crosslinking (grey).

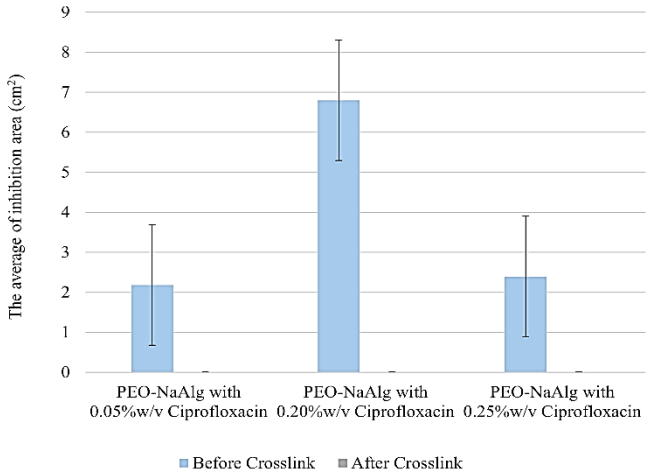

Figure 13. The average inhibition area of electrospun containing ciprofloxacin against *S. aureus*, before crosslinking (blue) and after crosslinking (grey).

The inhibition zone was observed on nutrient agar spread *E. coli* and *S. aureus*, placing a 6 mm diameter sample of ciprofloxacin electrospun both before and after crosslinking. The inhibition area that appeared around the electrospun samples was measured and averaged, as shown in Figure 12-13 of *E. coli* and *S. aureus*, respectively. The results showed that electrospun samples containing 0.05%w/v and 0.20%w/v ciprofloxacin before crosslinking effectively inhibit *E. coli* and *S. aureus* growth. However, after crosslinking, electrospun fibers loaded with both amounts of ciprofloxacin could inhibit *E. coli* but could not inhibit *S. aureus*. Since the fiber loading of 0.20%w/v ciprofloxacin ineffectively inhibited *S. aureus*, an attempt to improve the antibacterial activity of electrospun was performed by increasing the dosage of ciprofloxacin, up to 0.25%w/v. At 0.25%w/v ciprofloxacin. Before crosslinking, electrospun nanofibers successfully inhibited both *E. coli* and *S. aureus,* as shown in Figure 12-13, respectively. However, after crosslinking, even up to 0.25%w/v of ciprofloxacin effectively inhibits *E. coli* but still ineffectively inhibits *S. aureus*. However, the antibacterial efficiency of the electrospun fiber did not proportionally increase with increasing the concentration of ciprofloxacin loaded due to several causes, including the drug-polymer interaction, crystallization of the drug in the fiber, particularly at higher concentrations of drug leading to the larger drug crystal affecting the fiber morphology and the diffusion of the drug from the dense polymer network [51,52]. Among these three ciprofloxacin concentrations evaluated, the minimum concentration of 0.05%w/v in electrospun before crosslinking was sufficient to inhibit both *E. coli* and *S. aureus*.

However, the average inhibition zones for both bacteria were significantly reduced in the post-crosslinked electrospun samples. The inhibition zone of the crosslinked electrospun samples was observed and measurable only against *E. coli.* These results revealed that the crosslinking process affects the antibacterial properties of the electrospun material. Ciprofloxacin is a water-soluble antibiotic. $CaCl_2$ crosslinking of the alginate matrix can restrict drug diffusion and release, thereby reducing the apparent antibacterial activity, as reported by A. Kyziol [14]. The presence of Ca^{2+} ions can interact with ciprofloxacin, leading to complex formation and reduced drug mobility, which decreases its effective antibacterial performance [53]. Increasing the ciprofloxacin loading enhances the interaction with Ca^{2+} ions within the crosslinked matrix, resulting in the restriction of drug release and consequently reducing the antibacterial effectiveness, as reported previously by Amiruddin *et al.* [54]. Consequently, ciprofloxacin remained entrapped within the fibers after crosslinking. This approach has been employed to control the drug release rate in drug delivery systems [49,55]. However, the ciprofloxacin diffused from the crosslinked electrospun was insufficient to inhibit the growth of *S. aureus.* Ciprofloxacin is more effective against *E. coli* than *S. aureus* due to the differences in the mechanism of ciprofloxacin inhibition. In *E. coli*, ciprofloxacin inhibits both DNA gyrase and topoisomerase IV, which are the primary targets against the growth of *E. coli* [56]. However, in *S. aureus*, ciprofloxacin targets the topoisomerase IV as the primary target with high sensitivity, while DNA gyrase acts as a secondary target, and is less sensitive [57]. The resistance of ciprofloxacin in *S. aureus* occurs because the Nor A efflux pump is overexpressed to pump out the fluoroquinolones, therefore, *S. aureus* resists ciprofloxacin more effectively than *E. coli* [58]. It has also been reported that a higher concentration of ciprofloxacin is required to inhibit *S. aureus* compared to *E. coli* [59,60].

The electrospun nanofiber developed in this research is one of the essential materials for various biomedical applications. This polymer is biocompatible and biodegradable, making it suitable for various biomedical fields, and the material is cost-effective [17,61]. This research successfully fabricated PEO-NaAlg electrospun nanofibers based on a low molecular weight of 200 kDa to 300 kDa of PEO. The results reveal that the viscosity of the PEO-NaAlg solution affects the size and morphology of fibers. Water insolubility of the polymer was achieved using the crosslinking method. After loading ciprofloxacin, the electrospun nanofiber was effective against the growth of *E. coli.* However, it was insufficient to inhibit *S. aureus* because of its resistance to ciprofloxacin. To address the limitation of electrospun nanofibers from PEO-NaAlg, improving the antibacterial polymer by increasing the concentration of drugs, studying the release mechanism, and investigating other antibiotic drugs will be explored in further work. Since the achievement of E. coli inhibition of this fiber, it could also be applied scaffold in tissue engineering or filtration systems trapping the bacteria, which requires biocompatibility and biodegradability of materials with antibacterial properties [62,63].

4. Conclusion

Based on the PEO at low molecular weight range between 200 kDa to 300 kDa, 7%w/v is the optimal concentration of PEO that can achieve the electrospun nanofiber formation. The stabilization of nanofibers was examined by adding 1%w/v of NaAlg with viscosities of 150 cP to 730 cP and conducting the crosslinking by replacing Ca^{2+} and Na^{+}. The electrospun nanofibers retained their fibrous morphology despite changes in solution viscosity caused by NaAlg and the post-crosslinking process. Additional antibacterial nanofiber was investigated by loading ciprofloxacin at 0.05%w/v to 0.25%w/v. Adding ciprofloxacin did not affect the morphology of the fiber. After crosslinking, the average diameter of the nanofiber with ciprofloxacin increased due to swelling from the addition of this agent. The PEO-NaAlg electrospun nanofiber loaded with 0.05%w/v ciprofloxacin could efficiently inhibit *E. coli* and *S. aureus*. However, the crosslinking process reduces the effectiveness of *E. coli* and *S. aureus* inhibition. Further optimization will be needed for its effective application in future work.

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