

THE PROPERTIES OF CHITOSAN NANOEMULSION/ POLY (VINYL ALCOHOL) NANOCOMPOSITE MEMBRANES FOR WOUND CARE TREATMENT

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Abstract

The influence of chitosan nano-emulsion (CSNe) concentrations on the properties of CSNe reinforced poly (vinyl alcohol) (PVA) nanocomposites is the aim of this study. Polymer solution of CSNe with different concentrations combined with PVA having a low (PVA_L) and high (PVA_H) molecular weight (Mw), respectively, was fabricated to be the nanocomposite membranes by an electrospinning technique. The morphological characterization of the nanofiber formed in the membranes and the chemical group of PVA and chitosan analysis was performed by scanning electron microscopy (SEM) and Fourier- transform infrared spectroscopy (FTIR), respectively. The tensile properties of all the membrane specimens were tested according to the ASTM D-882. The neat-PVA_L and CSNe/PVA_L nanocomposite membranes were subjected to the in Vivo test of the open incision on mice. The results showed that an increase of CSNe concentration decreased the viscosity of CSNe-PVA polymer solution but increased the electrical conductivity. Those conditions reduced the nanofiber diameter with various fiber structures. Membranes used PVA_L yielded in higher tensile strength and lower tensile modulus than those used PVA_H. In Vivo test results of the CSNe/PVA_L membrane showed acceptable effectiveness in the wound care treatment to the Sofra-tulle commercial membrane containing antibiotics, and both were better than the neat-PVA membrane. Thus, the CSNe/PVA_L provides a promising chance for an alternative wound cover membrane.

Keywords: Chitosan nano-emulsion (CSNe), Electrospinning, Nanocomposite membrane, PVA, Tensile properties, Wound dressing.

1. Introduction

Chitosan (CS) with a chemical formula of $(C_6H_{11}NO_4)_n$ has been used in various industrial applications such as agriculture, biomedical, and food industries. Their biocompatibility, biodegradability, non-toxicity, antimicrobial in nature, and wound healing effect made the chitosan potential for biomedical use [1, 2]. The properties have dramatically increased research interest worldwide to fabricate fibrous membranes with a synthetic poly (vinyl alcohol) (PVA) by electrospinning method for wound dressing and drug delivery [3-5]. PVA was selected as one of the conductive polymers that are also non-toxic and possesses biocompatibility and easily blended with chitosan as the natural polymer [6].

A study using the electrospinning technique involves solution and processing parameters. Solution parameters include concentration, viscosity, molecular weight, surface tension, and conductivity. The effects of the parameters on the morphology of the fibrous membrane were studied [7-9]. It is known that the nanofibers produced by electrospinning method are in the form of a non-woven membrane with randomly oriented fibers whose diameter typically ranging from 100 nm to 500 nm or submicron [10, 11].

Higher viscosity produced unbeaded fiber structure. Higher net charge density revealed unbeaded and smaller fibers. Changes in mechanical properties of the nanofiber membrane are attributed to the fiber diameter and the formation of beads. The smaller the fiber diameter, the higher the tensile strength and tensile modulus, which lead to being the lower the ductility of the membrane. Beads were considered as fiber-defect that have a correlation effect with the concentration. An increase of the concentration of the polymer solution to a sufficient level leads to inhibit the formation of beaded fibers, which can improve the tensile strength of the membranes [12-14].

The electrical conductivity of the polymer solution has provided an effect on the average fiber diameter. The higher the electrical conductivity, the smaller the fiber diameter [9]. In the electrospinning process, there is a relationship between jet current, charge density at the fluid surface, and applied voltage. If the electrical conductivity of the polymer solution is too low, there are not enough charges in the solution to generate the Taylor cone. As a result, the fiber is not formed. Inversely, if the electrical conductivity of the polymer solution is enough, it would enhance the charges density on the fluid surface, which leads to the formation of a Taylor cone on the spinneret tip. This condition causes the electrospinning process to generate the fibers at the beginning. The enhancing of electrical conductivity beyond the critical value leads to hinder the Taylor cone and electrospinning [15].

The polymer solution parameters had been applied to the study of CS/PVA based fibrous membranes, which were mostly used for blended chitosan and PVA with high molecular weight (Mw). A recent study had succeeded in manufacturing CS/PVA fiber mats with continuous and uniform fibers resulted from CS/PVA ratios of 20:80 and 15:85, using 2% of CS in acetic acid and high Mw PVA of 85,300 g/mol. The electrospinning process was operated at an applied voltage of 15 kV and 20 kV [16]. However, other studies have used a similar concentration of the CS solution, Mw of PVA (85,000-124,000 g/mol), and applied voltage in the electrospinning process ranging from 21 kV to 25 kV, Paipitak et al. [17] and Biazar et al. [18] produced quite different fiber morphologies. The beaded-fibers were mostly formed [17], but severe

beads were not formed, although the solution and operating conditions were similar [18]. Another study [19] of CS/PVA used the same CS concentration, but slightly lower Mw of PVA (72,000 g/mol) than those previously mentioned studies, CS/PVA ratio of 10:90, and electrospinning process was operated at the same applied voltage as reported by Gonçalves et al. [16]. These conditions yielded unbeaded-fibers. On the other hand, Islam et al. [5] had investigated the blended CS/PVA nanofiber films by varying the CS concentrations and use very high Mw of PVA (146,000-186,000 g/mol). The physical and mechanical properties of the CS/PVA nanofiber films with and without TEOS as a chemical cross-linking were discussed as a function of CS concentrations. The addition of TEOS seemed to significantly improve the tensile strength and Young modulus.

Studies of CS/PVA nanofiber membranes fabricated using high Mw of PVA (89,000-98,000 g/mol) [13], 85,300 g/mol [16], 85,000-124,000 g/mol [18], 80,000 g/mol [20] and 146,000-186,000 g/mol [5] with various CS/PVA ratios showed the tensile strength ranging from 4 MPa to 11 MPa. Among those studies, the highest tensile strength (~11 MPa) was yielded from the CS/PVA membrane; being added with the chemical cross-linking [5]. Without chemical cross-linking, however, the tensile strength of the membrane significantly reduced to be less than 4 MPa. Related to the application of the nanofiber membrane for wound dressing, high tensile properties: i.e., tensile strength and strain, need to be possessed by the membrane, but the high tensile modulus is not necessary because it tends to be brittle. For instance, if the membrane is used to cover the incision occurred on a knee, and when the foot moves from straight to a bent position, the membrane will receive a tensile load. Thus, the membrane with those properties is required.

In this research, the use of CS Nano-emulsion (CSNe) is expected to improve the mechanical property of the CSNe/PVA nanocomposite membranes as a challenge for generating the fibrous membrane innovation. Besides, the research of CS/PVA fibrous mat by replacing CS solution with CSNe has not been carried out. The addition of CSNe may be able to control the viscosity and the electrical conductivity of the CSNe/PVA solution. In this work, therefore, the various concentrations of CSNe were blended with a low and high Mw of PVA, respectively. Effects of CSNe concentrations and Mw of PVA on the properties of CSNe reinforced PVA nanocomposite membranes were discussed. The tensile properties of the CSNe/PVA nanocomposite membranes were compared to those of CS/PVA membranes resulted from the earlier studies. The selected CSNe/PVA nanocomposite membrane was used for wound care treatment through the in Vivo test on the mice, which were then compared to the neat-PVA and the commercial membranes containing antibiotics of Sofra-tulle.

2. Experimental Methods

2.1. Materials

Two kinds of PVA were used in this work. A technical grade PVA (Goshenol, PVOH/PVA) with low molecular weight (Mw) of 22000 g/mole was purchased at CV. Multi Kimia, Yogyakarta, Indonesia, and a high molecular weight of PVA (85,000-124,000 g/mole) from Sigma Aldrich (USA). Chitosan micro powder, sodium tripolyphosphate (STTP), sodium hydroxide (NaOH), and acetic acid (CH₃COOH) were purchased from Sigma Aldrich (USA). Distilled water was used as a solvent in all experiments.

2.2. Preparation of CSNe and polymer solution of CSNe/PVA

Five gram of CS micro-powder was dissolved in 1000 ml of 1 % CH₃COOH and mixed by stirring on a magnetic stirrer, then kept for 24 h. Next, 1200 ml of 1 % CH₃COOH and 20 ml of 0.5 % STTP were gradually added into the CS-CH₃COOH solution (0.5 % w/v) also by stirring on a magnetic stirrer and then kept for 24 h. In this case, the addition of CH₃COOH was carried out first. Those produced CSNe with an inhomogeneous size of the particles: i.e., CS nanoparticles located on the top level, which was separated from chitosan micro-particles precipitated at the bottom.

The preparation of the polymer solution was started with the preparation of a PVA solution. Both high Mw and low Mw of PVA were prepared similarly. Ten gram of PVA was dissolved into 100 ml distilled water while being heated at 90 °C with stirring continues for one hour on a magnetic stirrer, resulting in the PVA solution of 10 % (w/v). The spinning solution was prepared by mixing the CSNe and PVA solution at room temperature and magnetically stirred for one hour. The concentration of CSNe_PVA solutions was varied, viz. 0 %, 5 %, 10 % and 15 % (w/w).

2.3. Fabrication and characterization of CSNe/PVA nanocomposite membranes

The CSNe_PVA solutions with various CSNe concentrations of 0 %, 5 %, 10 % and 15 % were electrospun at room temperature. The electrospinning process was carried out under the optimized conditions of the applied DC voltage of 15 kV, a flow rate of 0.025 ml/min, a spinneret diameter of 0.7 mm, and fix distance from tip to the collector plate (TCD) of 15 cm. Each solution concentration needed about three hours to produce the nanocomposite membranes with a thickness ranging from >50 μm to <100 μm, which was measured from the cross-section of the membrane using an optical microscope (Start Stram Olympus, BX53M). In this work, there are eight specimens, which are designated as CSNe-0/PVA_H, CSNe-5/PVA_H, CSNe-10/PVA_H, CSNe-15/PVA_H, and CSNe-0/PVA_L, CSNe-5/PVA_L, CSNe-10/PVA_L, CSNe-15/PVA_L. PVA_H and PVA_L are high and low Mw of PVA, respectively.

The transparent CSNe/PVA nanocomposite membranes were subjected to Fourier-transform infrared (FTIR, Shimadzu) spectroscopy examination to characterize the existence of specific chemical groups of PVA and chitosan. For FTIR analysis, the membrane specimens were prepared as thin as possible to make a transparent membrane: i.e., by shortening the electrospinning process for about five minutes.

The tensile test was carried out on all membrane specimens, which were prepared following the ASTM D-882, and about six samples were made for each testing variation. The tensile tests were carried out using a testing machine (Zwick Z0.5 Germany) at a crosshead speed of 10 mm/min and a gauge length of 20 mm.

Morphological characterization by scanning electron microscopy (SEM, TESCAN VEGA3 LMU, HITACHI SU-3500) was carried out on the entire specimen areas. Before being inserted into the SEM specimen chamber, the surface of the nanocomposite specimen was metallic-coated with an Au-Pd alloy. The fiber diameter or fiber size of the nanocomposite membrane observed in the SEM image

was measured for at least 100 fibers using the ImageJ open-source software. Besides, small amounts of CS nanoemulsion were subjected to transmission electron microscopy (TEM, JEM-1400) to observe the morphology of CS nanoparticles. The size of CS nanoparticles was predicted from a bright-field TEM image of CS nanoparticles morphology. An electron diffraction pattern taken from the CS nanoparticles area was used to identify the phase of CSNe.

2.4. Preparation of in Vivo test

The in Vivo test in this work used 15 mice aged 2-3 months and an average weight of 200-250 grams. Before testing, the mice were treated for adapting the environment for at least five days. They were then divided into three groups called treatment, negative control, and positive control. Each group used 15 % chitosan (CSNe-15/PVA) membranes for treatment K(P): i.e., the negative control group K(-) utilized neat-PVA membranes, and the positive control group K(+) employed a standard antibiotic gauze membrane.

Each mouse was subsequently anesthetized with chloroform to create a 2 cm diameter circular incision on each mice back that had previously been shaved (Fig. 1). Then, the treatments were carried out every three days for seventeen days by observing the rate of the healing of the incisions based on changes in wound diameter, the formation of wound scabs, and wound moisture. The dimension of nanocomposite membranes used for the wound dressing was 3 cm x 4 cm. In this research, CSNe-15/PVAL was chosen as a representative membrane, because it contains the highest CSNe concentration and has a proper value of modulus of elasticity. During the treatment, the incision was covered by the membrane as shown in Fig. 2.



Fig. 1. Photograph of an open incision made on the mice.



Fig. 2. The incision treatment with a CSNe-15/PVAL nanocomposite membrane.

3. Results and Discussion

3.1. FTIR analysis

FTIR spectra (Fig. 3) demonstrate a broad-band between $3600 - 3100 \text{ cm}^{-1}$, which are related to the O-H and N-H stretching vibrations. C-H stretching is shown at

the peak position around 2925 cm^{-1} . The bands at 1635 , 1543 , and 1026 cm^{-1} indicate the presence of amide I, II, and weak amide III bonds, respectively.

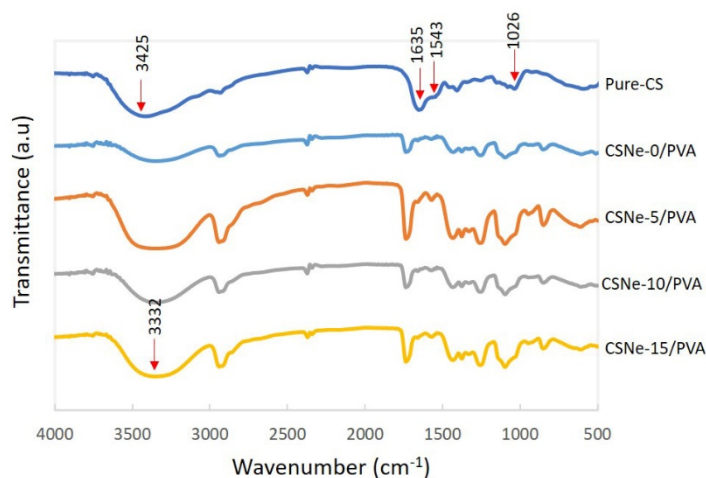


Fig. 3. FTIR spectra of pure CS, neat-PVA and CSNe/PVA nanocomposite membranes in the range of 4000-500 ($1/\text{cm}$).

The main peaks of PVA identified at 3332 , 2916 , 1720 , 1427 , 1324 , 1095 and 833 cm^{-1} , are assigned to O-H stretching vibrations from hydroxyl groups, asymmetric C-H stretching vibrations, C = O stretching of carbonyl, C-H bending vibrations of CH_2 , C-H deformation vibrations, stretching C of the acetyl group, and C-C stretching vibrations, respectively.

The graph shows the increasing intensity of the band at around 3332 cm^{-1} , due to an increase in CSNe concentration in the nanocomposite membranes, which occurs in the -OH stretching vibrations of the PVA with the secondary chitosan group of -NH [5, 16]. An increase of CSNe content in the membrane increased the intensity of the C-H band at 1720 cm^{-1} . Likewise, at the peak of 1082 cm^{-1} , an increase of the CSNe concentration increased the intensity of the C-O vibrational stretch. There is a peak at wavenumber 1087 cm^{-1} , indicating the wavenumber of the bond between C-O in the secondary alcohol found in chitosan and PVA. It can also be seen that there are adjacent peaks at 1026 cm^{-1} due to C-O in the primary alcohol contained in chitosan.

3.2. Fiber morphology

SEM micrographs of the CSNe/PVA nanocomposite membranes fabricated using PVA with high Mw (Fig. 4) and low Mw (Fig. 5) by varying CSNe concentrations produced a various fiber size and structure feature of fibers. An increase of CSNe

Concentration leads to a reduction in the average fiber size (Fig. 4). This is not seen in the nanocomposite membranes prepared using PVA with low Mw (Fig. 5). The formation of beaded-fibers with a high-volume fraction of beads occurred in a membrane made of PVA with a high Mw at 10% CSNe concentration (see arrows in Fig. 4(c)), although the fiber size was the smallest. Based on the bead-shape, the stretched droplet beads [7] were predominantly formed in this specimen with a

distance between the beads. Therefore, the probability of building cross-linking between fibers would be possible (Fig. 4(c)). Although the electrospun nanofiber mat with the presence of beads is usually considered as poor quality [21], it may depend on the number, shape, and position between them.

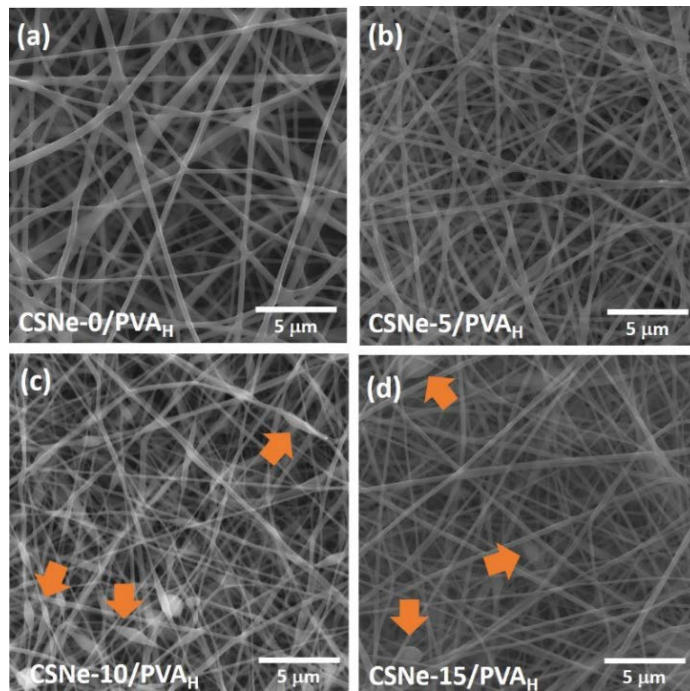


Fig. 4. SEM micrographs of CSNe/PVA_H nanocomposite membranes showing the formation of beads marked by arrows.

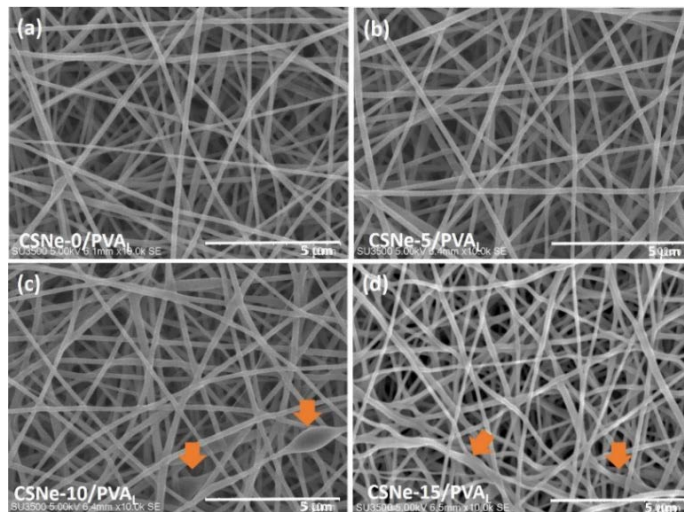


Fig. 5. SEM micrographs of CSNe/PVA_L nanocomposite membranes showing the formation of beads marked by arrows.

The small fiber size formed in this specimen was due to changes in viscosity and electrical conductivity of the CSNe-10_PVA spinning solution. Unlike the CSNe/PVA_H nanocomposite membranes, the formation of the bead was almost not observed in the CSNe/PVA_L membranes, which is related to the viscosity of the spinning solution. The addition of CSNe concentration into the PVA with high Mw produced higher solution viscosity than PVA with low Mw (Table 1).

The polymer with high Mw has longer molecular chains than the low Mw does. It provided an effect on the processing of polymer fibers formation [9, 22]. Thus, at similar applied voltage, the structural feature of the membrane made of PVA with low Mw produced almost bead-free fibers. Besides, an increase of CSNe concentration increases the electrical conductivity of the spinning solution, leading to the formation of higher charge density on the surface of the electrospun jet. As a result, the repulsive forces between the polymer chains become stronger, and the fiber diameter become smaller [20].

The relationship between the physical properties of the spinning solution: i.e., the viscosity, electrical conductivity, and the fiber size obtained from this study showed a similar trend to previous studies [8, 9, 12, 23]. In this study, changes in fiber size and structure due to the viscosity and electrical conductivity of the spinning solution are closely associated with the Mw of PVA. The correlation effect of those factors on the mechanical properties of the CSNe/PVA nanocomposite membranes is discussed in the following section.

Table 1. Viscosity and electrical conductivity of the CSNe PVA spinning solution, and related fiber diameter.

Specimen	Viscosity (cP)	Electrical conductivity ($\mu\text{s}/\text{cm}$)	Average fiber diameter (nm)
High Mw of PVA (Mw: 85.000 - 124.000 g/mole)			
CSNe-0/PHA _H	872.35	1388.00	265
CSNe-5/PHA _H	602.90	1392.33	234
CSNe-10/PHA _H	441.90	1394.67	144
CSNe-15/PHA _H	325.90	1426.33	165
Low Mw of PVA (Mw: 22.000 g/mole)			
CSNe-0/PHA _L	577.90	539.67	197
CSNe-5/PHA _L	369.90	590.00	192
CSNe-10/PHA _L	313.90	613.33	169
CSNe-15/PHA _L	218.90	651.67	141

3.3. Tensile properties

The previous findings [9, 23] have reported that an increase of Mw of the polymer solution enhanced the tensile strength and modulus of the electrospun nanofiber mats. In this work, the tensile strength of PVA with high Mw is lower than that of PVA with low Mw, which is inconsistent with others [9, 23], whereas the tensile modulus showed the opposite (Fig. 6).

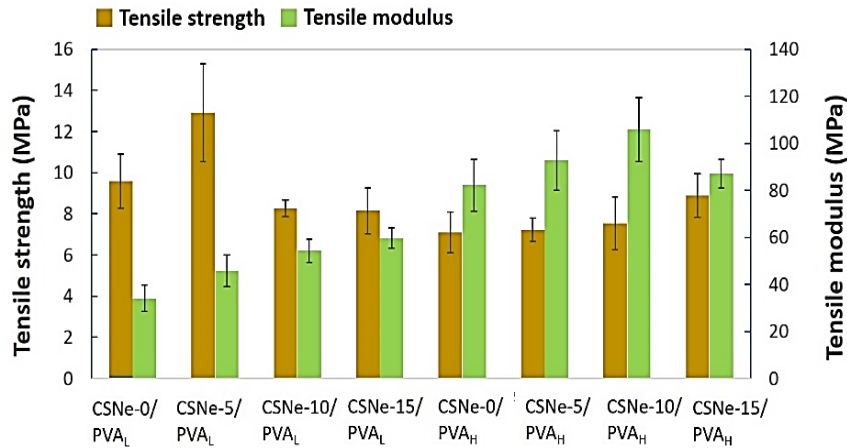


Fig. 6. Tensile strength and modulus of CSNe/PVA nanocomposite membranes.

The tensile strength and modulus of the CSNe/PVA membranes showed different values with those of nanocomposite membranes fabricated using PVA with high Mw and low Mw. The differences in Mw of neat-PVA (CSNe-0/PVA) exhibit a similar trend. Thus, Mw of the polymer has played an important role in affecting the properties of the produced electrospun nanofiber membranes.

The tensile strength of nanocomposite membranes prepared with high Mw of PVA improved linearly with CSNe concentration, but not in the membranes with low Mw of PVA. The addition of 5 % CSNe concentration drastically increased the tensile strength up to 12.9 MPa and reduced significantly with the addition of 10 % and 15 % CSNe concentrations.

These results confirmed that the tensile strength of the electrospun membrane is not only influenced by the fiber diameter, but also the formation of cross-link between fibers. It might be caused by the high-volume fraction of the cross-link between fibers. Cross-link between fibers observed in the SEM micrographs is not corresponding to the addition of the chemical cross-linking in the CS/PVA blended solution [5, 20], which usually aims to increase the mechanical property of the produced nanofiber membranes. It should be noted that the addition of CSNe into the PVA solution showed the best achievement in improving the tensile strength compared to the other CS-based PVA fibrous membranes [5, 13, 16, 18, 20].

Another significant point is the presence of CSNe in an amorphous phase, as confirmed by the TEM examination (Fig. 7). It is shown that CS nanoparticles tend to agglomerate (Fig. 7(a)) due to its hygroscopic property. The particle size is around 50 nm (Fig. 7(b)). An electron diffraction pattern (Fig. 7(c)) obtained from a region comprising of the CS nanoparticles indicated the existence of diffuse intensity without diffracted spots, explaining that CSNe is present in an amorphous phase.

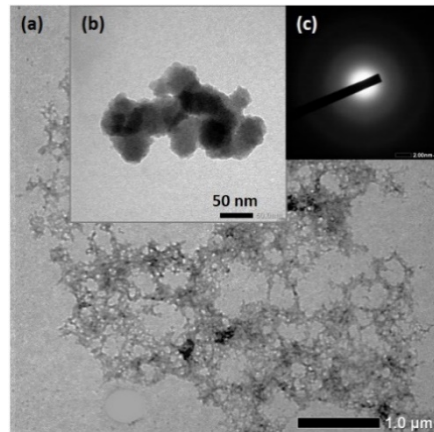


Fig. 7. TEM images of CSNe. (a) A low magnification image, (b) a magnified image and (c) an electron diffraction image related to the CSNe particles showing an amorphous phase.

The incorporation of CSNe and PVA was more effective than blended CS and PVA in enhancing the tensile property of the electrospun CS/PVA nanofiber, although the CSNe is present in an amorphous phase. The tensile modulus of all nanocomposite membranes in this study was comparatively high, especially those fabricated using PVA with high Mw (Fig. 6), which are not favourable for wound dressing application. Figure 8 shows the elongation at break of all nanocomposite membranes in which the highest was achieved by a membrane with 5 % CsNe using PVA with low Mw. The higher the CSNe concentration, the lower the elongation at break by using PVA with either high or low Mw.

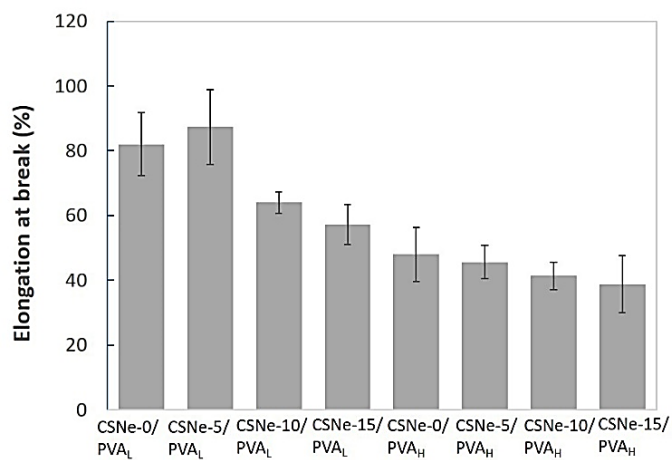


Fig. 8. Elongation at break of CSNe/PVA nanocomposite membranes.

It is suggested that the proper tensile properties for wound dressing are those included in the range of the native skin properties: i.e., 5 - 30 MPa for tensile strength, 4.6 - 20 MPa for tensile modulus and 35 - 115 % for elongation at break

[24]. Therefore, the membranes prepared with low Mw PVA are better than those with high Mw PVA in terms of the tensile strength and elongation.

The comparison of the tensile properties of the CSNe/PVA membranes (the present results) and those of the CS/PVA membranes using CS_PVA blended solutions (previous studies) has been summarized in Table 2. These results underlined that the addition of CSNe improved the tensile strength and elongation at break of the CSNe/PVA, especially using low Mw PVA, although that was without adding the chemical cross-linking. The tensile strength and elongation at break of CSNe-5/PVA_L (present work) are higher than those resulted in previous works (Table 2). Thus, the improvement of the tensile strength by adding CSNe concentration might be due to the uniformity of agglomerated CS particles along the fibers. This increased the volume fraction of cross-link between fibers and reduced the fiber diameter, as shown in Table 1.

Table 2. Comparison of the tensile properties of the CSNe/PVA and CS/PVA nanofiber membranes.

Membranes	Mw PVA (g/mol)	Tensile Strength	Tensile Modulus	Elongation at break	References
CSNe/PVA _L (5% CSNe)	22,000	12.91 MPa	45.83 MPa	87.29%	Present study
CSNe/PVA _H (5% CSNe)	85,000-124,000	7.22 MPa	92.77 MPa	45.64%	Present study
CS/PVA (30/70)	89,000-98,000	6.5 MPa	190.4 MPa	4.5%	[13]
CS/PVA (30/70) (15/85)	85,300	4.15 MPa 6.13 MPa	201.7 MPa 94.2 MPa	3.96% 14.13%	[16]
CS/PVA (20/80)	85,000-124,000	< 2 MPa	-	22%	[18]
CS/PVA with chemical cross-linking	146,000-186,000	11.2 MPa	570 MPa	25%	[5]
without chemical cross-linking		4 MPa	300 MPa	25.3%	
CS/PVA with chemical cross-linking	80,000	8 MPa	107.9 MPa	23.9%	[20]
without chemical cross-linking		3.8 MPa	36.5 MPa	30.8%	

The evolution of fiber structure related to the improvement of tensile properties would be interesting to be discussed if both the polymer matrix and fillers are present in the crystalline phase. Interfacial bonding between the filler and the

matrix can be examined from the high-resolution TEM (HRTEM) image or lattice image which may be able to study the mechanism of stress-strain at the interface as reported by Levitas and Roy [25, 26] who had investigated the application of theory for temperature and stress-induced phase transformation in two-variant martensitic nanostructures. Therefore, the mechanism of tensile properties of the nanofiber membranes should be clarified in the next research by using the semi-crystalline polymer reinforced with the metallic nanoparticle.

3.4. Application of the CSNe/PVA nanocomposite membrane for the wound care treatment

This preliminary study applied the nanocomposite membrane produced by electrospinning and developed for wound healing and wound dressing. Observation of the effectiveness of the chitosan wound cover membranes for healing the incisions in the in vivo test was carried out macroscopically by measuring the diameter of the incisions on the 2nd, 5th, 8th, 11th, and 17th days. The measurement was carried out on all the 15 mice tested in this study. The percentages of healing of the incisions were measured at each observation period based on changes in their respective individual diameters, as shown in Fig. 8. The results (Fig. 8) indicate that the progression of the healing of incisions was significantly different among the three treatment groups on the fifth day and the seventeenth day. The progress of the healing occurred from the fifth day with a long time to heal until the seventeenth day. However, the development of the healing of the incisions using the CSNe-15/PVA_L membrane was as good as the one using the antibiotic gauze membrane (Sofra-tulle dressing). Compared to the negative control group, it exposed different results on the fifth day and seventeenth day.

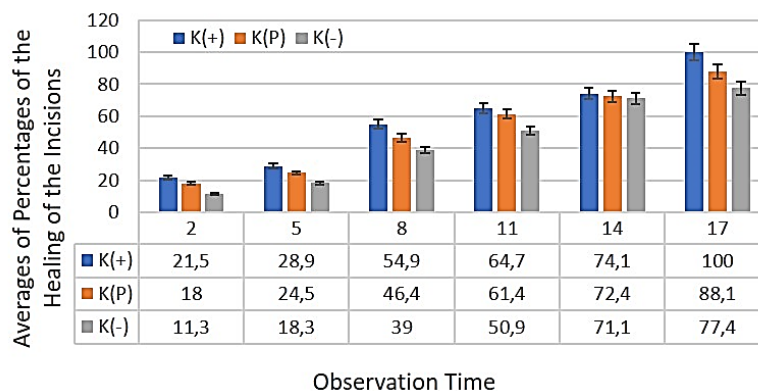


Fig. 8. Progressivity of time-based healing of the incisions.

The formation of scabs around the incisions characterizes the healing process. In this study, the scabs were peeled when they had piled up because the membrane application does not work optimally. Observation of the formation of scabs revealed that the positive control group had a faster healing time compared to the treatment group and the negative control group.

The incisions moisture-content parameter has three classifications, namely wet, moist, and dry incisions. The observation of incisions moisture disclosed that on the eighth day, the treatment group and the positive control group had a dry incision

on all tested mice. It means that these two groups had the same speed in drying the incisions, whereas all tested mice in the negative control group showed merely dry incision after the fourteenth day of treatment.

Although the wound healing process using the chitosan membrane does not achieve the maximum result compared to the antibiotic gauze membrane, the healing effectiveness reached nearly 90 %. It was also clearly shown that the addition of CSNe into the PVA resulted in better wound healing than the neat-PVA. The photographs of the wound healing on the mice on the eighth day, fourteenth day, and seventeenth day are exhibited in Fig. 9.

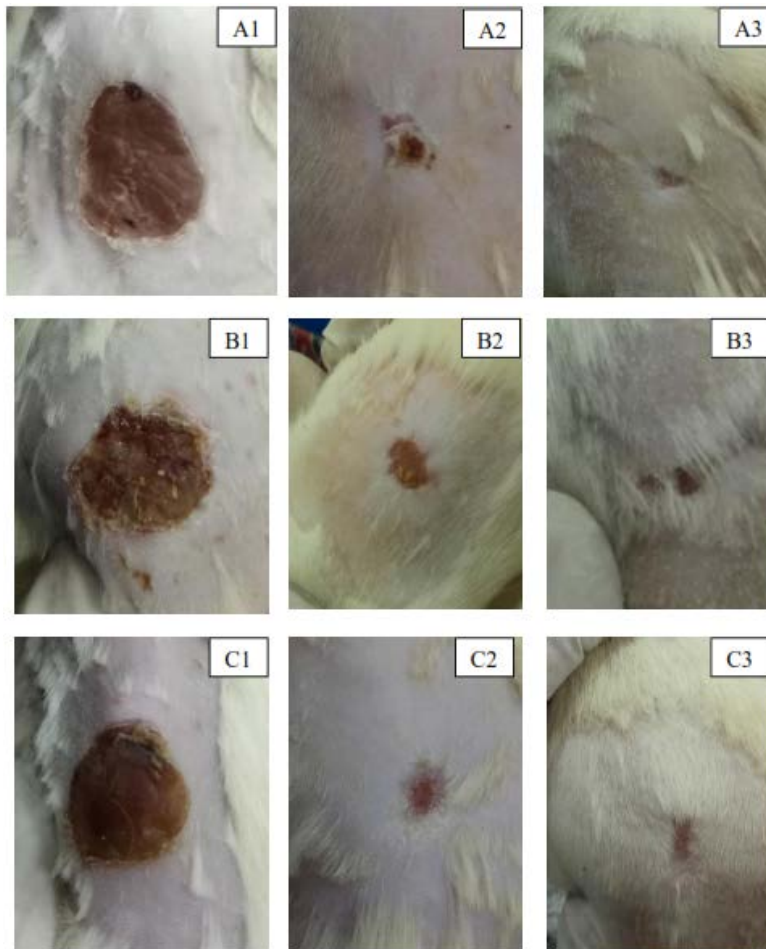


Fig. 9. Photographs of wound healing process in the eighth day (A1, B1, C1), fourteenth day (A2, B2, C2) and seventeenth day (A3, B3, C3). A1-A3, B1-B3 and C1-C3 are the healing process using CSNe-15/PVA, neat-PVA and Sofra-tulle membranes, respectively.

Chitosan is an active compound obtained through the process of demineralization, deproteinization, and deacetylation of natural materials, such as shellfish, crabs, or shrimp shells [27]. The process of wound healing is quite

complicated and dynamic due to many biological processes involved in it. The results of this study correspond to the theory of wound healing, where the progression of wound healing occurs in the first five days of the inflammatory phase. The difference in the progress of wound healing on the fifth day in the three groups in this study might be due to differences in membrane content. The treatment group (CSNe-15/PVA_L) showed the same results as the positive control group. The results of the study are also in line with the research of Chhabra et al. [28], which states that chitosan can initiate the division of fibroblast cells and stimulate collagen synthesis that is essential in the wound healing process.

5. Conclusions

This study has demonstrated a significant role of CSNe incorporation with PVA in which CSNe was present as an amorphous phase. The addition of 5 % of CSNe into the PVA solution (PVA with low Mw: 22000 g/mol) had shown a valuable finding in the achievement of the comparatively high tensile strength of CSNe/PVA_L nanocomposite membrane to be ~ 13.00 MPa. The tensile modulus and elongation of the membrane were 45.83 MPa and 87.30 %, respectively. The CSNe-15/PVA_L had also exhibited similar effectiveness to the standard antibiotic gauze membrane as a wound cover membrane, and better than the neat-PVA_L membrane. It is suggested that the CSNe/PVA_L membrane could be developed as an alternative wound cover membrane. Besides, this result would be a useful contribution to a comparative study in the bio-engineering research field.

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Abbreviations

CS	Chitosan
CSNe	Chitosan Nanoemulsion
PVA	Poly (Vinyl Alcohol)
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy

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