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The Fabrication and Characterization of Electrospun PVA-Snail Mucin Nanofiber Membrane

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ABSTRACT

Snail mucin (SM) has been reported containing antimicrobial properties. This electrospun PVA-SM solution research aims to develop and characterize the nanofiber membrane for wound healing application. The PVA-SM solutions with various concentrations (1%, 3%, 5%, and 7% (w/w)) were prepared by mixing the PVA, distilled water, and SM extracted from a local snail. The PVA-SM solutions were then electrospun at an optimized parameter. The morphology and tensile properties of the nanofiber membrane fabricated were examined using scanning electron microscopy (SEM) and universal testing machine. The nanofiber size diameter was measured using image analysis software. *Results*: PVA-SM nanofiber membrane bead-free was successfully fabricated. Nanofiber diameter with high uniformity was found in the range of 100-300 nm. The SM concentration of 3% resulted in the smallest nanofiber diameter with an average diameter of 190 ±28.7 nm leading to the highest tensile strength (6.5 MPa), yet its elastic modulus was still higher than that of the natural skin. Whereas the other SM concentrations produced lower tensile strengths achieving around half of the one. In this work, the relation of the polymer solutions properties with the resulted nanofiber membrane was evaluated and explained. Based on the tensile properties, the fabricated nanofiber membrane is potentially used in biomedical applications.

Key words: Snail mucin, Nanofiber, Electrospinning, Natural fiber

1. INTRODUCTION

Over the last two decades, electrospinning is known as an outstanding technique to produce nanofibers from composites, polymers, and ceramics [1]. This method is a very straightforward, versatile, cost-effective and flexible prepared process [2]. In principle, the electrospinning process involves a high voltage being applied on a contained polymer solution to generate a high electrostatic field leading to the ejection of a continuous jet of solution that gets accelerated from the spinneret towards a grounded target as a collector. Nanofibrous membranes fabricated by the technique have been reported presenting excellent characteristics, such as high interconnected porosity, gas permeation, high surface area to volume ratio, and dequate mechanical stability [3]. These properties promote electrospun membrane ideal candidates for various applications, e.g., protective clothing [4], filtration [5,6], and biomedical [7]. Especially biomedical applications, the membrane is able to prevent microbial infiltration, maintain moisture balance and exchange air with the environment, distribute antibacterial, encourage cell proliferation in wounds, and have good biocompatibility [8]. Thus, the electrospun nanofiber membrane could potentially offer superior environmental for wound healing.

Natural biopolymers such as cellulose, silk fibroin, chitosan, hyaluronic cellulose gelatin, and collagen have been electrospun into nanofiber membrane for biomedical applications [9]. However, the utilization of protein-based polymers suffers from rapid degradation rates in vivo and low mechanical stiffness. To overcome these weaknesses, synthetic polymers were electrospun. Various synthetic polymers commonly applied for nanofiber membrane in biomedical application include poly(ethylene oxide) (PEO) [10], poly (ε-caprolactone) (PCL) [11], poly(lactic-*co*-glycolic acid) (PLGA) [12] polyvinylpyrrolidone (PVP) [13], polyurethane (PU) [14], poly(lactide) (PLA) and poly(vinyl alcohol) (PVA) [8]. Among those, the last is known as a biodegradable polymer that is biologically friendly due to its superior properties such as non-toxic, non-carcinogenic, biocompatible and possessing the appropriate mechanical properties. The polymer also shows flexibility and expandability in aqueous media so that PVA is developed extensively as a wound dressing [2,15]. In addition, the use of electrospun bioresorbable synthetic polymer may reduce the number of bandage replacement.

Antibacterial and anti-inflammatory agents, whether metallic, inorganic or organic could be added to the polymeric nanofibers to impede bacterial colonization, following wound disease, thereby accelerating wound healing [16]. The silver

nanoparticles (AgNPs) are one of the antimicrobial agents having been reported for treating wound infections [7]. However, the utilization of chemical compounds for wound healing may result in poisonous effects in *in vitro* research, thus encouraging scientists to use natural antibacterial and anti-inflammatory agents (non-synthetic). Aloe vera and chitosan are known as natural polymers having shown the activity of microbial. They have been mixed with PVA and electrospun as a nanofiber membrane for a potential wound dressing material [17]. The researchers also successfully developed a PVA polymer solution blended with aloe vera and chitosan to produce the nanofiber membrane [18]. Snail mucin (SM), another natural polymer, has also been confirmed comprising antimicrobial proteins [19] having biological value in inhibition of inflammatory process and wound healing, resulting in doubled faster for healing time than the normal saline solution.

SM has abundantly been produced from snail (*Achatinafulica*) mucous secretion. In order to obtain the benefits, composite membranes of PVA-alginate-snail mucin with fiber size in the range of 100-300 nanometers have been developed [20]. Beads have been mostly found in the membrane being produced. The fewer beads were observed at the higher voltage. The electric field does not successfully draw the resulting beads occurring from PVA-alginate snail slime polymer due to an imbalance between the electric voltage and the polymer flow rate. From the analysis of the functional groups of the PVA-alginate snail slime composite using the FTIR, the five composite samples with snail slime addition showed the not much different infrared absorption results. It indicates the identities of the compound produced and the absence of the formation of new functional groups. Yu et al., [21] fabricated nanofibers with snail extract powder as additives by the electrospinning and the bubble electrospinning. Unsmooth morphology of nanofibers indicating a high surface-to-volume ratio with an average diameter of 255 nm has been observed. In addition, the nanofibers also present high alkali-proof and acid-proof properties. However, those investigates have not reported their mechanical properties. Nugroho et al., [22] have successfully manufactured the PVA-SM nanofiber membrane bead-free utilizing the electrospinning method with fiber size diameter in the range of 200-900 nanometer. The addition of SM into the polymer solution influences the fiber morphology leading to affect mechanical properties of the membrane. However, the effect of the SM addition to the physical properties of the polymer solution has not been observed yet, and this tensile strength is still lower than that of the commercial microfibrous dressing.

Based on those reviews, there is still room to study PVA-SM membrane nanofiber. The goal of this work is to study the influence of SM concentration on the PVA-SM nanofiber membrane fabricated by using electrospinning technique and their properties, including viscosity and conductivity of the polymer solution, morphology, and tensile properties of the membrane.

There area some other nanomaterials research with different application [23-25].

2. EXPERIMENTAL METHOD

2.1. Material preparation

PVA used in this research was commercially acquired from PVA Gohsenol powder (PVOH/PVA with the molecular weight of 22000gr/mol), the SM was extracted from 20-25 local snails. The mucin was then filtered for contaminant removal and stored into the refrigerator. PVA solution was prepared by fully dissolving 10 gr of PVA powder in 100 ml distilled water under continuous magnetic stirring for an hour at a temperature of 80^{o} C. The 10 % (w/v) PVA solution was then allowed to cool to room temperature. Afterward, various SM concentrations in SM-PVA solution, i.e., 1%, 3%, 5%, and 7 % (w/w) were prepared by blending the SM liquids in PVA solution (10% v/w) using magnetic stirrer with 200-300 rpm at ambient temperature for three hours. The viscosity and conductivity of each concentration of SM-PVA solutions were then determined using a viscometer (Brookfield Viscometer) and conductometry method.

2.2. Membrane fabrication

Nanofiber membrane samples were fabricated by electrospinning the four different aqueous polymer solutions described above at the optimized parameters set up, comprising tip to collector distance (TCD), and voltage. A spinneret with a diameter of 0.6 mm as the electrode linked to the power supply was used during the optimization process. The SM-PVA solutions were filled in a 5 ml syringe equipped with stainless steel spinneret at the nozzle and a 20 gauge scale. The electrospinning process was carried out at an optimized voltage of 15 kV at room temperature. The applied voltage created a jet of the solution to be drawn as nanofiber spun toward a 25 x 25 cm aluminum foil wrapped on a grounded flatten collector. The aluminum foil was located at an optimized distance of 16.5 cm away from the spinneret tip – in other words, TCD = 16.5 cm. The SM-PVA solutions were supplied at a measured flow rate of around 0.025 mL/minute by a syringe pump. To obtain the desired thickness in the range of 40-60 μm, the fabrication was carried out for three hours for each membrane at room temperature. A typically fabricated nanofiber membrane is shown in Figure 1. Lastly, the nanofiber membrane was carefully detached from the aluminum foil collector and stored in a desiccator.

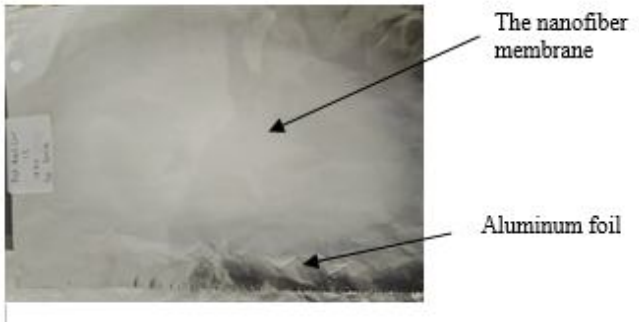

Figure 1: The fabricated nanofiber membrane before detached from aluminum foil collector

2.3. Characterization

Randomly selected nanofiber membrane was cut into square form for microscopy characterizations. An optical microscope (OM, Olympus BX53M) was employed to measure the cross-section thickness of the membrane. While a scanning electron microscopy (SEM, JSM-6510LA) was applied to investigate the fiber formation and morphology. Before a nanofiber membrane was located in the chamber, its surface was metallic coated with Au-Pd alloy. The fiber size diameter of the nanofiber membrane was determined by random measurement of two different regions of each membrane by using an image analysis open-source software, ImageJ from National Institutes of Health, USA. A Universal Testing Machine type of Zwick Z0.5 Germany was utilized to evaluate the tensile properties of the nanofiber membrane. It was equipped with a 100 N load cell and a gauge length of 20 mm, and loaded at a crosshead speed of 5 mm/min. The tensile testing procedure was prepared in accordance with ASTM D882. The prepared specimen [22] was depicted in Figure 2.

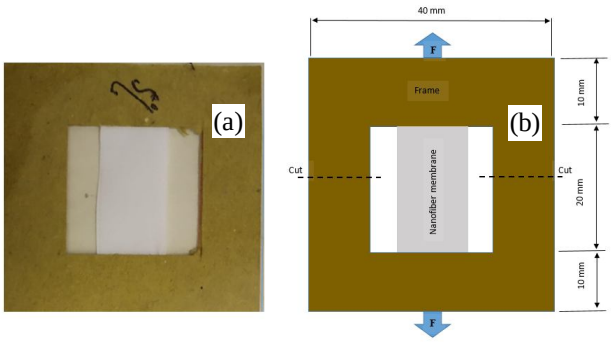

Figure 2: (a) Tensile test specimen and, (b) dimension of the specimen holder prepared in accordance with ASTM D882

3. RESULTS AND DISCUSSION

3.1. Morphology

During the electrospinning process, the size and shape of the nanofiber are affected by some parameters such as concentration, viscosity, surface tension and conductivity of the polymer solution. Those parameters are contributed by the solvent [26]. Those parameters could be altered by varying the solvent composition at a certain composition of the solution.

The concentration of aqueous PVA solution chosen in this work was at 10% (w/v) due to its capability to produce nanofiber as reported elsewhere [22]. Even though the parameters are important, conductivity and viscosity of polymer are the most significant parameters influencing the final characteristic of the electrospun fiber [5]. Hence, before processing the electrospinning, the properties of the solution were evaluated and presented in Table 1. The addition of SM as a charge carrier for instance salt or conductive filler possessed an effect on the conductive of the polymer solution leading to an increase of the conductivity of the solution with the rise in SM concentration. After being observed, the viscosity of the polymer solution was discovered to change in a small range due to increased concentration.

Table 1: Conductivity and viscosity of PVA-SM solutions at various concentrations

Specimen	SM Concentration % (w/w)	Viscosity (cP)	Conductivity (µS/cm)
PVA-SM-1	1%	477,9	617,67
PVA-SM-3	3%	455,9	715,67
PVA-SM 5	5%	405,9	827,33
PVA-SM 7	7%	479,9	908,33

The SEM micrographs of the nanofiber membranes fabricated by those parameter processes were illustrated in Figure 3. It is clearly observed that the fibers are a bead-free and finely spun fibrous structure with the appearance of continuous linear and in random orientation. The fibers also show homogenous in size diameter alongside with their lengths. The SM concentrations might be included in an appropriate parameter causing the solvent to evaporate more easily, resulting in an acceptable nanofiber structure [27]. In addition, the nanofiber membrane appears to have high interconnected porosity in nanometer size due to the presence of intersecting nanofiber structure and overlapping each other, allowing to facilitate air and moisture to exchange to wound. Additionally, the structure effectively inhibits the penetration of bacteria. SM concentrations of the polymer solutions generally gave a small effect on the morphology of nanofiber in less concentration. Figure 3(a) and 3(b) show nanofiber at 1% and 3% SM concentrations, which seem to be similar. However, at the higher SM concentrations, Figure 3(c) and 3(d), the difference between them is more apparent to be observed. Nevertheless, all the figures show that the neat structure of the membranes has been successfully fabricated.

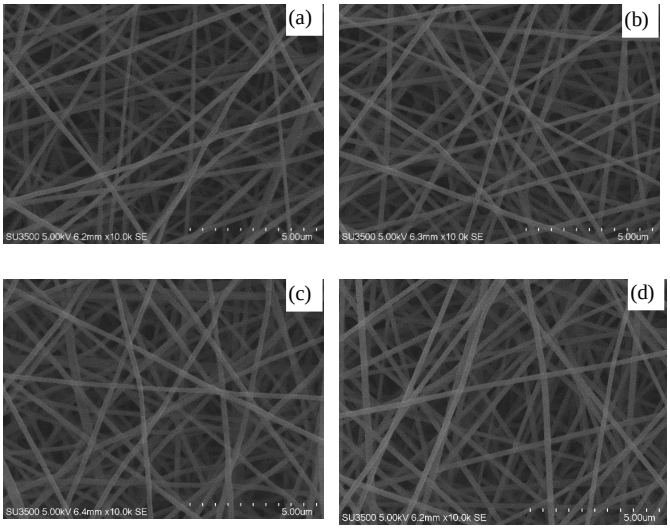

Figure 3: SEM micrograph of PBA-SM nanofiber membranes at varous SM concentrations: (a) 1%, (b) 3%, (c) 5% dan (d) 7%.

In order to confirm the observation result of the surface morphology on the SEM micrographs as presented by Figure 3, the measurement of the fiber diameters using the ImageJ digital image analysis software was conducted. The distribution of fiber diameter is shown in Figure 4. The nanofiber membranes with SM concentration of 1% and 3% present considerable similar fiber distribution. Mostly (>80%) were found in the range of 151-250 nm, with average diameters of 209 ± 29.8 nm and 190± 28.7 nm, respectively.

However, the latter was observed more uniformly because of the more percentage of nanofiber accumulated at the range size diameter of 151-200 nm, as shown by Figure 4(a) and 4(b). On the other hand, at higher concentrations of SM (5% and 7%) the diameter of the nanofiber was shifted to the right where the fiber diameter was predominantly found in the range of 251-300 nm with average diameter of 274,2 ± 55.1 and 238,2 ± 29,9 nm, respectively as depicted in Figure 4(c) and 4(d). Overall, the diameter of the membranes fabricated in this study is comparable to that of the previous studies [20,21], which is in the range of 100-300 nm. It has been reported elsewhere that PVA-SM concentration 0% produced a membrane with fiber diameter, tensile strength, and elastic modulus of 460.17 ± 128.32 nm, 3,48 MPa, and 12.00 MPa, respectively [22].

Regarding this, the addition of SM concentration mostly reduced the fiber diameter. The addition up to 3 % resulted in a noticeable decrease in average nanofiber size diameter but an increase in the uniformity of the nanofiber membrane. Furthermore, an increase of SM concentrations at 5 % and 7 % increased the fiber mean diameter and reduced uniformity.

The altering viscosity of polymer solution because of the SM addition might contribute to the alteration in fiber mean diameter. In general, the viscosity of the spinning solution was associated with the degree of polymer molecular chains. A higher viscosity leads to a large fiber diameter obtained due to a higher viscoelastic resulting in difficulty of jet splitting [28]. Due to not prominent viscosity changes caused by the increase of SM concentration, then the average diameter changing of the fiber was also not too large. The conductivity of the polymer solution acted a significant effect during starting the electrospinning process.

The conductivity of polymer solution increased from 617, 67 μS/cm to 715,67 μS with an increase of the SM concentration from 1% to 3% resulting in a slight decrease of the electrospun nanofiber. Although for SM concentration from 3% to 5 %, it was predicted that decrease viscosity could yield a decrease in fiber diameter, SEM micrograph measurement showed the opposite result. During the electrospinning process, these main parameters are influencing and interacting with one another, and the polymer solution conductivity predominantly affects the process than that of viscosity [18].

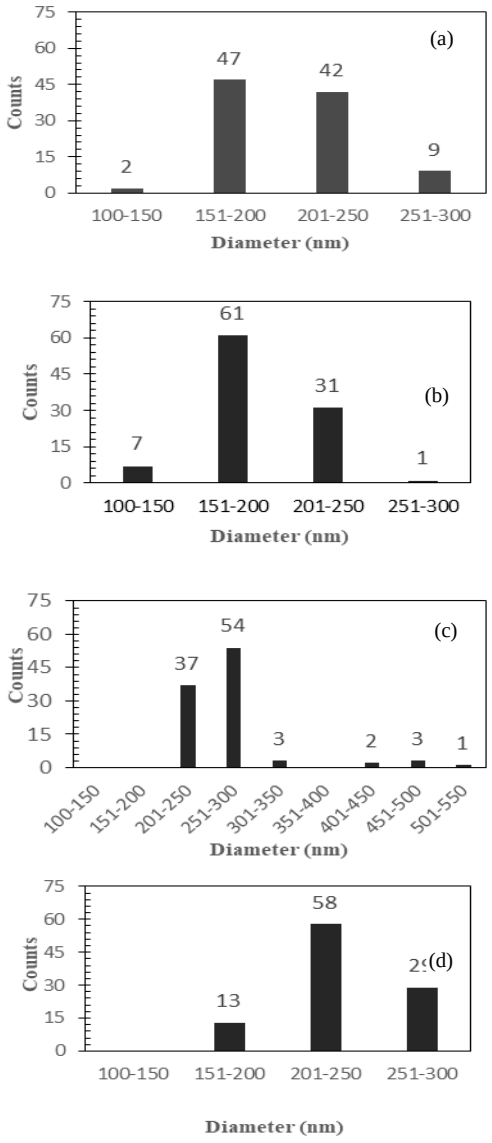

Figure 4: Distribution of nanofiber membrane size diameter at various SM concentrations: (a) 1%, (b) 3%, (c) 5% dan (d) 7%.

3.2. Tensile properties

This work proposes to fabricate the electrospun nanofiber membrane possessing appropriate tensile properties for biomedical applications, including tensile strength, elastic modulus, and elongation at break, with the values in the range of 5.00-30.00 MPa., 4.60-20.00 MPa, and 35.00-115.00 % respectively. Herein, the tensile properties of the electrospun PVA-SM nanofiber membrane with various SM concentrations were examined and presented in Figure 5. A typical stress-strain curve of polymeric material was demonstrated by the stress-strain curve of the membranes indicating most curve shape at loading region and large elongation as depicted by Figure 5(a). The effect of SM concentration might change the behavior of their stress-strain curve, including the tensile strength, and the elastic modulus, as illustrated in Figure 5(b). The relationship between tensile strength and elastic modulus of the PVA-SM nanofiber membrane revealed a comparable tendency.

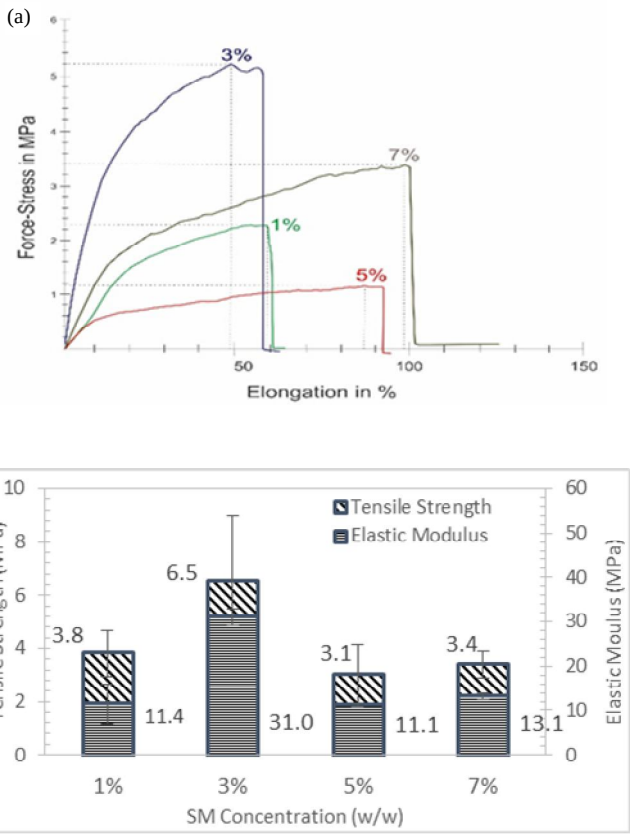

Figure 5: (a) Forces-stress versus elongation of the membrane at each SM concentration, (b) Tensile strength and elastic modulus of the membrane at each SM concentration

The SM concentration of 3% attained the highest tensile strength at 6,5 MPa due to its smallest nanofiber diameter obtained, followed by that of 1 %, 7%, and 5% with the tensile strength of 3,8 MPa, 3,4 MPa, and 3,0 MPa, respectively. It could be associated with the alteration in the morphology of the nanofiber membrane. The tensile strength and elastic modulus were found to increase with the decrease of fiber size [29]. When the fiber size diameter decreased, a surface area of each volume unit of fiber increased, and the contact point between fibers also enhanced, leading to expanding fiber network, which offered a good effect on a higher elastic modulus and tensile strength. In addition, Figure 5(a) also exhibits the correlation between tensile strength and elongation at break.

All of the nanofiber membranes demonstrated large elongation at break in the range of 59 – 123 %, with the SM concentration 5% showing the largest. It indicates the high flexibility of the membrane and the high mobility of the PVA chain [30].

These results exhibit that the tensile properties could be controlled by choosing a suitable parameter. These findings indicate that the PVA-SM nanofiber membrane at 3% concentration has tensile strength and the elongation at the break closing to those of the natural skin, yet the elastic modulus is slightly higher than that of the natural skin. On the other hand, at the other SM concentrations, only their elastic modulus match to that of the requirement. Therefore, further optimization of the processing parameter should be conducted to achieve the appropriate properties of the native skin in future research.

4. CONCLUSION

In the present work, the effect of solution concentration at the optimized parameter on the electrospinning process along with morphology and tensile properties of the nanofiber membrane being fabricated were all evaluated. The conclusion can be drawn as follows:

1. All fabricated nanofiber membranes had no fibrous bead structure with a smooth surface and homogenous.

2. The addition of SM concentration of 3% reduced the fiber diameter to 190 nm leading to obtaining the highest tensile strength (6.5 MPa) and elongation at break of 87.5 % MPa, which were able to be included in the range of native skin. The elastic modulus, however, higher than that of natural skin. On the other hand, the other SM concentrations produced nanofiber membranes with the tensile properties in the opposite ways.

3. This work also confirms that the concentration affects the viscosity and conductivity of the polymer solution, which performs an important role in controlling the quality of the fiber morphology and the tensile properties of the nanofiber membranes being fabricated.

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