

Engineering Physics International Conference, EPIC 2016

Polyvinyl Alcohol/Soursop Leaves Extract Composite Nanofibers Synthesized Using Electrospinning Technique and Their Potential as Antibacterial Wound Dressing

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Abstract

The ethanolic extract of soursop leaves (SLE) has been proven to have antibacterial activity and can be used to cure some bacterial diseases caused by *Staphylococcus aureus*, *Escherchia coli*, *Enterobacter aerogenes* and it also has the ability to heal skin infections. In this study, SLE incorporated with polyvinyl alcohol (PVA) as matrix polymer produced fibers by electrospinning process. Electrospinning is one of the techniques used in producing fibers up to nano size and applicable used as wound dressing. Wound dressing with fiber appearance and contained antibacterial agent was very important concerned in protection utterly bacterial and and the ability to synthesize protein better in the wound healing process. Composite fiber PVA/SLE synthesised from PVA solution and added SLE with 8-14 % weight ratio into solution. This study showed: (1) successfully producing electrospun PVA/SLE composite fiber with average fiber diameter 121-137 nm, (2) process parameter for solution PVA/SLE spinned with voltage 8 kV, flow rate 0.2 mL/h and distance from collector to injection was 12 cm and from the in vitro test of antibacterial activity, the composite nanofibers were confirmed to be able to halt the growth of *Staphylococcus aureus* implying that the composite nanofibers could be applied as a good wound dressing material.

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Peer-review under responsibility of the organizing committee of the Engineering Physics International Conference 2016

Keywords: Soursop leaves extract; Polyvinyl alcohol; Electrospinning; Antibacterial wound dressing

1. Introduction

The immediate dressing of wound is the cornerstone of wound healing management. Ease of application and bio adhesiveness to the wound surface, sufficient permeability of water vapor, ease of sterilization and a good barrier to bacteria are the essential parameters required for ideal wound dressing [1]. The prevention of infection caused by bacteria is an important factor for wound healing process. In recent years, some research groups have tried to produce many different nanomaterials for faster healing including the development of nanofibers with antibacterial agents [2, 3]. The diameter size of nanofibers ranging between 500 nm to 1 mm with large surface area is very efficient for wound dressing to protect the wound from bacterial penetration and is extremely capable of fluid absorption and dermal delivery [1].

We have recently reported the development of nanofibers membranes by an electrospinning system with drum collector for wound dressing applications. The system could produce a nanofibers membrane with a large surface area so that active substances in the nanofibers could freely interact with skin surface for faster wound healing while the thickness of the membrane could be controlled by changing the voltage and flow rate [4]. Moreover, the electrospinning technique is used to produce nanofibers with flexible and superior mechanical performance (e.g. stiffness and tensile strength). Therefore, it is a

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suitable method to produce nanofibers mats for applications in tissue engineering [5]. Detailed information about the electrospinning method and its process parameters including the flow rate, applied voltage, nozzle to collector distance, and viscosity are described in some previous works [6-8].

In this study, a wound dressing system with an antibacterial activity, which was composite nanofibers with polyvinyl alcohol (PVA) as the polymeric matrix loaded by soursop leaves extract (SLE), was produced by the electrospinning technique. Soursop, or *Annona muricata* L. is a species of neotropical fruit that can be consumed directly or after being processed. It was reported that SLE has an antimicrobial activity that resists the growth of some bacteria such as *Pseudomonas aureginosa*, *Staphylococcus aureus* and other bacteria that may cause skin infections [9, 10] Moreover, it contains sugar, anthraquinone, flavonoids, terpenoids, tannins, alkaloids, and cardiac glycosides [10]. Therefore, it is traditionally used for wound treatment and other medical applications [11]. On the other hand, the direct application of crude soursop extract onto a wound may cause infection because of microorganisms contained inside the extract [12]. Loading SLE with an antibacterial activity [9, 10] into PVA with non-toxicity, non-carcinogenicity, and bio-adhesive characteristics [2] will form an excellent layer of the extract on the surface of the polymeric matrix with emulsive and adhesive properties that can be advantageous for wound dressing application.

2. Methods

2.1. Materials

Fully hydrolyzed PVA (degree of hydrolysis > 98%, Mw = 26.500 g/mol) was purchased from Sigma-Aldrich. The dried soursop leaves were bought from a local market and distilled water was obtained from the Department of Chemistry, Institut Teknologi Bandung (ITB). For an antibacterial test, a nutrient agar medium, a nutrient broth medium, and *Staphylococcus aureus* bacteria were provided by the Bioremediation Laboratory, ITB.

2.1.1. Soursop leaves extract (SLE) solution preparation.

The simplex powder as much as 300 g of soursop leaves were macerated using ethanol as a solvent for 5 days in darkness and occasionally stirred. After 5 days, the macerates were then mixed together and concentrated by means of the rotary evaporator at the temperature not exceeding 40°C. The 10% (w/w) soursop leaves extract solution was prepared by dissolving 1.1 g of the concentrated solutions of the extract in 10 g ethanol (27°C) and stirred at 300 rpm to obtain a homogeneous solution.

2.1.2. PVA/ SLE solution preparation.

For preparing electrospun polymer matrix fibers, the fully hydrolyzed PVA was dissolved in ethanol to form 10% (w/w) PVA solution. To vary the concentration of the SLE in the final solution, 10% (w/w) SLE solution was added into PVA solution to make 8%, 12% and 14% (w/w) of PVA/SLE solution. A magnetic stirrer was applied for 2 hours at room temperature to achieve a homogeneous solution.

2.2. Thin Layer Chromatography (TLC)

Thin layer chromatography was performed to determine the existing bioactive chemical compounds in SLE, such as alkaloids, flavonoids, phenols and terpenoids, which can be separated by different polarities in TLC plates. The samples of SLE were spotted on the TLC plates. The TLC plate (2 cm × 10 cm) was made from silica gel GF-245 and put in contact with the solvent system of *n*-hexane: ethyl acetate: glacial acetate acid (3:6:1) v/v [4]. The spots were then analyzed under UV light at the wavenumbers of 254 nm and 366 nm. The chemical compounds were identified by the characteristics of colored spots after being sprayed by reagents system.

2.3. Electrospinning Process

The set of electrospinning apparatus is Nachriebe 600 Electrospinning, which was manufactured by Instrumentation and Materials Laboratory, Department of Physics, ITB. In order to prepare the PVA/SLE nanofibers, the electrospinning setup used in this study consisted of a syringe and a needle with an internal diameter of 0.84 mm, a collector plate made from aluminum, and a high voltage power supply. The syringe was mounted on a syringe pump to control the flow rate. The PVA/ SLE solution was electrospun at the voltage of 8 kV, the distance between the needle tip and the collector of 12 cm, and the flow rate of the solution of 0.2 mL/h. The electrospinning process was carried out for 6-8 hours at room temperature.

2.4. Scanning Electron Microscopy

To achieve accurate observation on the morphology and the size of the fibers, the membranes were investigated under a scanning electron microscope (SEM) (JEOL, JCM-6000 SEM NeoScope Benchtop) with an excitation voltage of 10 kV. The diameter of fibers was determined by measuring 100 points across each fiber in the SEM image. The measurement was done using *ImageMIF ver. 3.0* software (Dept. of Physics, ITB).

2.5. Fourier-Transform Infrared (FTIR) Spectroscopy

The FTIR analysis was performed to determine the existing functional groups in PVA nanofibers, SLE solutions, and PVA/SLE composite nanofibers. The scanning of wavenumbers was conducted within 500 to 3800 cm^{-1} . By referring to their peak numbers, the typical functional groups at each sample can be determined. Additionally, the information regarding about the structure of each compound can be gathered while the purity of each compound can be assessed.

2.6. The determination of in-vitro antibacterial activity

The assessment on the antibacterial activity of the nanofibers is very important for wound dressing applications. The antibacterial test was conducted based on a quantitative analysis using *Staphylococcus aureus* as the bacterial test strain. The gram-positive bacteria *Staphylococcus aureus* used in this study were obtained from the Bioremediation Laboratory, ITB. The bacterial suspensions were prepared by growing a single colony in a nutrient broth medium. A sterilized cotton swab was dipped into the suspension culture and the bacterial cells would spread homogeneously over the agar plates. The antibacterial activities of SLE solutions and PVA/ SLE composite nanofibers membranes (8%, 12%, and 14%) were then determined. Each sample was placed onto a sterilized membrane filter and placed onto an inoculated agar plate. The solution of SLE was used as a control. The samples and inoculated agar plates were incubated for 24 h at 37°C. The zone of inhibition (ZoI) was determined as the total diameter (mm) of PVA/SLE-filter paper. All measurements were performed in PVA/SLE with various concentrations and then compared with SLE solutions.

3. Results and Discussion

3.1 Chemical Compounds of SLE

The chemical compounds in the SLE were identified by thin-layer chromatographic (TLC) fingerprinting and profiling so the presence of bioactive compounds could be determined. When viewed under the UV light at 254 nm and 366 nm, there were four visible big spots on the TLC plate of the SLE. The bioactive compounds present in the extract were determined by specific spraying of reagents solutions. The illumination of the TLC plates under UV showed that the SLE depicted band patterns for alkaloids and flavonoids. The band patterns of phenols and terpenoids, which were identified by the previous study [10], did not exist as given in Table 1. The present findings differ with those obtained from the previous study because the type of solvent, the weight of leaves, drying method and extraction time differed and they would affect the chemical compounds in the SLE [14].

Table.1 The results of TLC identification of the SLE

Spray Reagent	Chemical components	Color reactions' Literature [13]	Color reactions' Result	Indication
Citroborate	Flavonoid	Yellow	Yellow	Presence of Flavonoid
Dragendorff	Alkaloid	Brown or orange-brown	Orange-brown	Presence of Alkaloid
Anisalhedid- H ₂ SO ₄	Terpenoid	Blue	Orange	Absence of Terpenoid
FeCl ₃	Phenol	Dark Blue	Green	Absence of Phenol

3.2. Electrospun Nanofibers

Macroscopically, the PVA/SLE composite nanofibers were stacked and accumulated to form a smooth surface, yellowish white membrane. At a higher concentration of the SLE, the yellow color of the membrane became more obvious accompanied with a stronger scent.

The morphologies of PVA/SLE composite nanofibers are shown by SEM images in Figure 1. It has been found that the average diameters of the PVA/SLE composite nanofibers made from the precursor solutions with SLE concentrations of 8, 12,

and 14% (w/w) were 137, 132, and 121 nm, respectively. This finding means that the higher the concentration of SLE or the lower the concentration of PVA, the smaller the average fibers diameter is obtained, which is in agreement with the previous work [16].

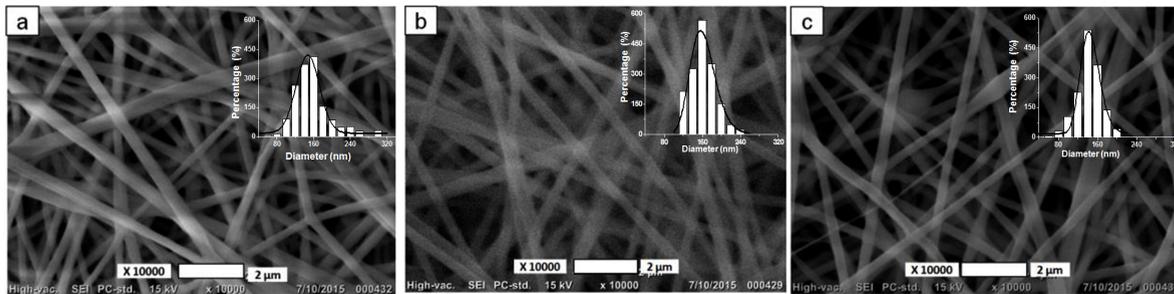


Fig.1. SEM images at 10,000× magnification and diameter distributions of PVA/SLE with the concentrations of SLE (a) 8%, (b) 12%, and (c) 14% (w/w)

3.3. FTIR Characteristics of the Nanofibers

The FTIR spectra of PVA nanofibers, SLE solution and PVA/SLE nanofibers are presented in Figure 2. The FTIR spectrum of PVA nanofibers gave multiple peaks at 1374 and 847 cm^{-1} representing C-H medium rocking as the alkanes group, 1249 and 1092 cm^{-1} for C-O strong stretching, and 1735 cm^{-1} due to C=O stretching as aldehydes group. In addition, there was a wide band in the range of 3200-3500 cm^{-1} with the peak at 3323 cm^{-1} and an additional peak at 2917 cm^{-1} due to the presence of a polyhydroxy group (O-H stretching). The FTIR spectrum of SLE solution showed peaks at 3326 cm^{-1} representing O-H stretching and 1380 cm^{-1} representing C-H stretching considered as alkaloid compounds [10]. Moreover, there were peaks at 2979, 1044 and 879 cm^{-1} which representing stretching of O-H, C-O, and C-H, respectively. Specific peaks of the FTIR spectra of PVA nanofibers and SLE solution were also observed in those of PVA/SLE composite nanofibers. Peaks of the amide I (1690-1760 cm^{-1} , C=O stretching) that are unique to PVA were still detected with larger absorbances when compared to the spectrum of PVA nanofibers. The peaks of O-H, N-H stretching and C-H, -NO₂ bending did exist in the PVA/SLE composite nanofibers within the same range of wavenumber as the SLE solution although with lower intensity. This lower intensity caused by the presence of PVA [15].

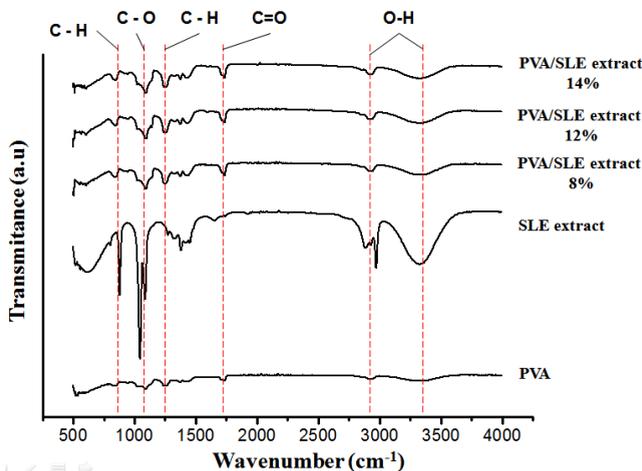


Fig.2. FTIR spectra of PVA nanofibers, SLE solution, and PVA/SLE composite nanofibers

3.4. Antibacterial Properties

The antibacterial activities of the PVA/SLE composite nanofibers and SLE solution were examined against Gram-positive *S. aureus*. The results indicated that the PVA/SLE composite nanofibers and SLE solution did have antibacterial activities. The ZoI diameter for Gram-positive *S. aureus* increased from 1 to 4 mm with increasing the concentration of PVA/SLE composite nanofibers. The results of ZoIs in the SLE solutions were fit with the earlier investigation that SLE solutions against *S.aureus*

[10]. The increase of extract concentrations will enhance their antibacterial activities caused by the increase of bioactive contents of the extract/compound [9]. The average diameter of the Zone of Inhibition (ZoI) of PVA/SLE composite nanofibers for *S. aureus* can be seen in Table 2.

Table 2. Diameters of zone of Inhibition (ZoI) for PVA/SLE composite nanofibers for different concentrations of SLE (8-14%) and SLE solutions

Concentration of PVA/SLE composite nanofibers (%)	ZoI Diameter (mm)
8	1
12	2
14	4
SLE solution	6

4. Conclusion

The present study successfully producing electrospun PVA/SLE composite fiber with average fiber diameter 121-137 nm, conclusively showed the presence of alkaloid and flavonoid groups on membrane due to FTIR spectrum analysed. This study also showed PVA/SLE composite fiber antibacterial activity against *S. aureus*. Antibacterial activities of composite fiber increased by increasing the concentration of PVA/SLE in composite nanofibers.

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