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6 **The Role of Relative Humidity on Physical Characteristics of Poly Vinyl Alcohol-Aloe vera Fiber Membrane by Using Electrospinning Methods**

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Abstract. Electrospinning is a method to fabricate nanofiber scaffold for tissue engineering. One of the parameters that affects the fiber size and morphology and the other physical characteristics is relative humidity of the room in which the electrospinning process is undertaken. The synthesis of electrospun Poly Vinyl Alcohol (PVA)-*Aloe vera* was performed with a variation of relative humidity (52%, 55%, 58%, and 64%). The physical characteristics of the fiber was tested by using functional group test, morphology test, thermal analysis test, and degradation test. The result of functional group test by using Fourier Transform Infrared (FTIR) showed that there was a crosslinking bond when the *Aloe vera* was introduced to the PVA. The morphology test result by using Scanning Electron Microscope (SEM) indicated that the addition of *Aloe vera* could increase the average of fiber diameter and the increase of relative humidity reduced the fiber diameter. The thermal analysis test result by using Differential Scanning Calorimetry (DSC) showed that the increase in the relative humidity could increase the temperature of glass transition (Tg) and decrease the temperature of melting (Tm). The degradation test implied that all the fibers could be degraded in 12 minutes.

Introduction

Electrospinning is a method to fabricate nanofiber [1]. The ability of electrospinning method to fabricate nanofiber is usually implemented in medical field as tissue engineering scaffold synthesis, wound dressing and drug delivery. Several parameters could affect the morphology and pore diameter on the fiber produced by electrospinning method. The parameters from the device, such as the high voltage, needle-collector distance, and flow rate and the parameters from the solution, like viscosity, solvent, and concentration have an important role in this method. Besides those two types of parameter, there is also another parameter that has a main role, which is environment, like temperature and humidity [1-3].

Humidity is one of the parameters that could affect the transformation of the polymer solution into nanofibers through electrospinning process. The humidity is defined as the water concentration in the air. The humidity that is studied in this research is the relative humidity. It is related to the hygroscopic properties of the polymer, which is important to know to make sure the water that could be trapped in the polymer and evaporation process. It is expected to form pores in which the cells could grow [4-6].

The relative humidity is correlated with the air temperature. In the afternoon, the temperature under the ground rises which declines the relative humidity on the environment. On contrary, in the night, the temperature under the ground behaves in the opposite condition. Medeiros et al. (2008) reported that when the relative humidity was 30%, the electrospinning result of polymer and its

solvent could be easily evaporated and formed small fibers and pores [4]. The nanofiber membrane from electrospinning process could be applied as tissue engineering or biomaterials. Several applications of membrane as wound dressing utilized *Aloe vera* to accelerate wound healing. *Aloe vera* is already used for more than 5000 years as a therapy for several diseases, such as joint inflammation, acne, skin inflammation and wound. Its function as anti-inflammation is suitable for wound dressing material. Polyvinyl alcohol (PVA) is known as a biomaterial that has been used for wound dressing because it is non-toxic, non-carcinogenic, moist and biocompatible [7-9]. Escobar-Sierra and Perea-Mesa (2017) reported in their study that PVA is biodegradable [10]. Abdullah et al. (2014) also reported that PVA could be used in the electrospinning process with the addition of *Aloe vera* [7]. Based on the information mentioned above, the study about the effect of relative humidity in electrospun PVA-*Aloe vera* nanofiber membrane was conducted with relative humidity between 52% and 64% on the functional group, the morphology, thermal analysis and degradation properties.

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Materials and Methods

Materials. The Polyvinyl alcohol (PVA) ($M_w = 66000$ gr/mol) was obtained from Duksan Pure Chemicals Co. Ltd. (Korea). The *Aloe vera* extract was purchased from Kangcare Bioindustry Co. Ltd. The other materials used were deionized water, ethanol, and Phosphate Buffer Saline (PBS).

Methods. PVA with concentration 10% w/v was dissolved in deionized water with a magnetic stirrer at temperature of 80°C for three hours. 5 w/v % *Aloe vera* extract was mixed to the PVA solution and put on top of magnetic stirrer with temperature of 50°C for three hours. The ethanol was added to the mixed solution of PVA-*Aloe vera* (70:30) and stirred using a magnetic stirrer for an hour to obtain a spinnable solution. The solutions were tested by using VT-04F RION Co. Ltd Viscotester to obtain the viscosity of the solution.

The electrospinning procedure was initiated with the preparation of the solution into the syringe throughout the needle tip. An aluminum foil was put on the collector section of the electrospinning machine to store the nanofiber membrane. The high voltage used in this study was 19 kV with 20 cm of needle-collector distance. The electrospinning process was carried out with several relative humidity (52%, 55%, 58% and 64%).

The characterization of nanofiber membrane was started with functional group test by using FTIR (Shimadzu). The FTIR test was carried out at wavenumber of 4000 - 400 cm^{-1} . The morphology of the electrospun PVA-*Aloe vera* based nanofiber membrane was characterized to observe the effect of different relative humidity by using Phenom Pro X Desktop SEM. The thermal analysis using DSC test was performed by using a METTLER TOLEDO DSC-3. The test was conducted by increasing the temperature from 27°C to 300°C with 10°C/min. The degradability test was performed by using 0.05 mL PBS, with observing the mass loss of a membrane with area of 2.25 cm^2 and mass of 0.01 gr overtime.

Result and Discussion

Morphology Test. The morphology of the electrospinning process of the PVA-*Aloe vera* membrane was shown in Fig. 1. The nanofiber membrane was white and microscopically, the fiber structure was thin and blurry. Fig. 1 showed that there was a part that looked like a layer inside the membrane. That could be originally from the membrane that was still wet. The solvent in this study was distilled water and the relative humidity in the environment of the electrospinning process would be affected by that. The presence of high amount of water content in the membrane would prevent the solution to form fibers [8, 11]. To observe more about the SEM result, the fiber diameter of each sample was measured and depicted in Fig. 2.

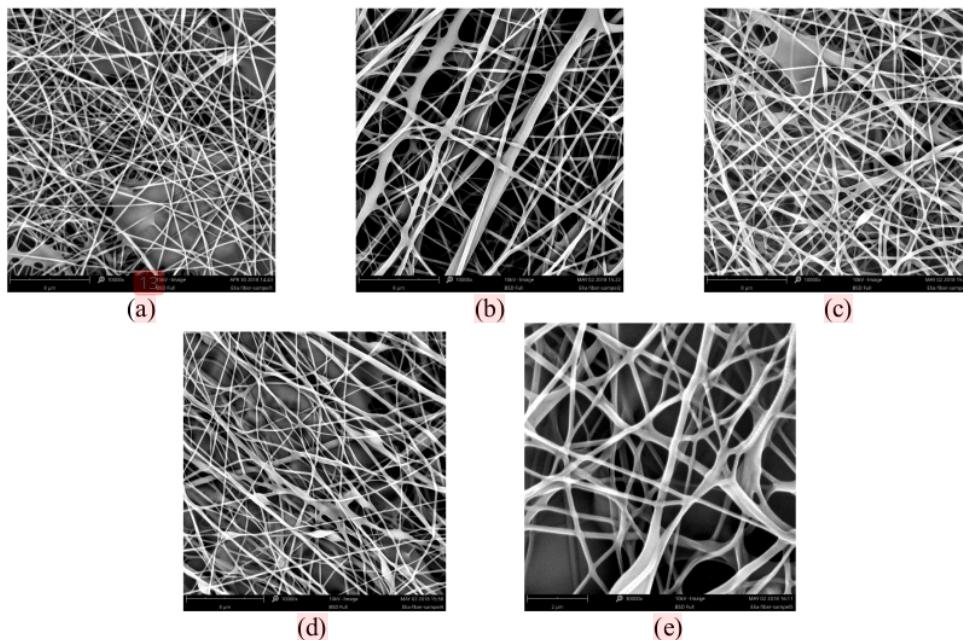


Fig. 1. SEM Images of the PVA-Aloe vera nanofiber membrane with several relative humidity
 (a)non Aloe vera RH 55% (b) RH 52% (c) RH 55% (d) RH 58% (e) RH 64%.

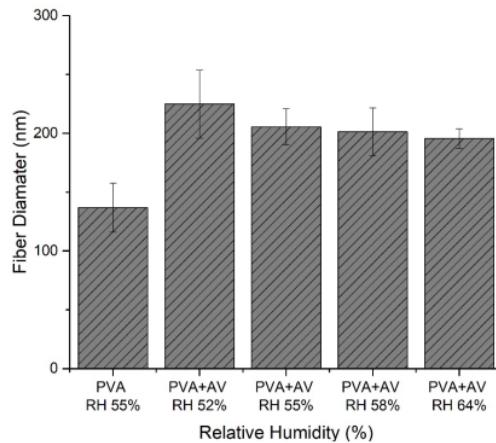


Fig. 2. Fiber Diameter of The PVA-AV Nanofiber Membrane with Several Relative Humidity.

The result in Fig. 2 showed that *Aloe vera* addition could increase the fiber diameter of the fiber membrane. Meanwhile, with the increase of the relative humidity in the environment of the sample synthesized, the fiber diameter of the sample decreased. When the samples with the same relative humidity was compared (with RH 55%), the sample with the addition of *Aloe vera* had bigger fiber diameter (202 nm). Overall, the addition of the *Aloe vera* in the sample with relative humidity between 52-64% increased the fiber diameter [4-5].

Functional Group Analysis. The result of functional group test of PVA, *Aloe vera* and PVA-*Aloe vera* was shown in Fig. 3. It showed that the hydroxyl functional group had an absorbance at wavenumber of 3309.15 cm^{-1} and 3310.70 cm^{-1} [12]. The stretching C-H functional group appeared at wavenumber of 2939.55 cm^{-1} , 2916.2 cm^{-1} and 2915.04 cm^{-1} for PVA, *Aloe vera* and PVA-*Aloe vera*, respectively. Besides that, they also had an absorbance at wavenumber of 1732.47 cm^{-1} and 1734.76 cm^{-1} which represented the stretching aldehyde (C=O) group. The peak at 1079.06 cm^{-1} and

1080.14 cm^{-1} indicated the C-O indicated the functional group of C-O stretching originated from the polyphenol group of *Aloe vera*. A stretching vibration of carboxyl and hydroxyl deformation was shown at 1374.33 cm^{-1} and 1374.04 cm^{-1} . This result implied that the membrane already contained *Aloe vera* extract [7-9].

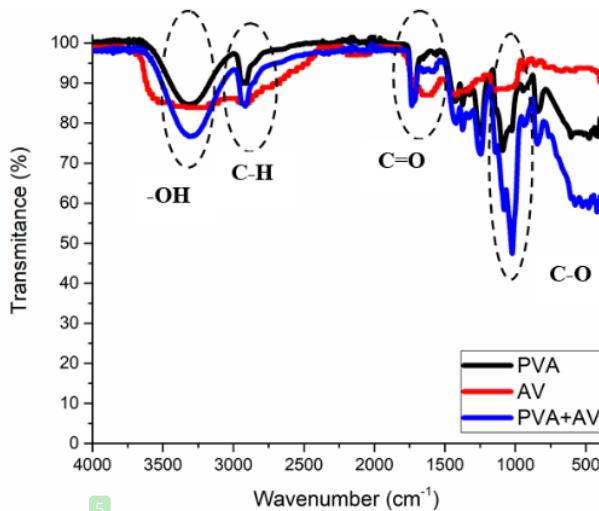


Fig. 3. FTIR Spectra of PVA, *Aloe vera* and PVA-*Aloe vera* nanofiber Membrane.

Thermal Analysis Test. Thermal analysis test by using DSC was performed to investigate the thermal properties of a sample. The thermal properties transition obtained was such as temperature of glass transition (T_g) and melting (T_m). By observing thermal properties, the information about the durability of the sample could be obtained and the storage management could be considered based on this information.

The result shown in Fig. 4 illustrated the thermal properties of nanofiber membrane sample with several relative humidity values. The glass transition temperature and melting temperature of the sample were different with the increase of relative humidity. The addition of *Aloe vera* in the PVA could increase the glass transition temperature which was from 85.05°C (PVA membrane with relative humidity of 55%) to 90.05°C (PVA-*Aloe vera* membrane with relative humidity of 52%) and 92.05°C (PVA-*Aloe vera* membrane with relative humidity of 55%). It still continued to increase at relative humidity of 58% and 64% which were 95.05°C. The low water content in the environment could accelerate the process to have an amorphous structure instead of crystalline structure. It also emphasized the result of fiber diameter of the nanofiber membrane with the increase of relative humidity. The lower relative humidity, the smaller the fiber diameter which indicated the acceleration to have amorphous structure [7, 8, 13].

The *Aloe vera* addition also increased the melting temperature. On contrary, the melting temperature (T_m) decreased with the increase of relative humidity from 52% to 64% on the sample with the addition of *Aloe vera* from 281.11°C to 187.23°C. The sample with relative humidity 52% had two endothermic curves which were 190.61°C and 281.11°C. The nanofiber membrane with smaller diameter would be easily melted compared to the bigger one. This result was appropriate with the study of Abdullah et al (2014) which reported the increase of melting temperature with the addition of *Aloe vera*. Generally, polymer solutions would form entanglement in their long chains which would lead to amorphous structure and thus lower the melting point of the membrane [8, 13].

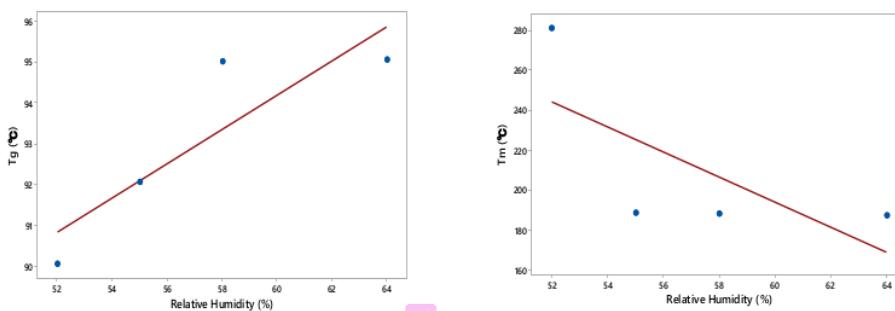


Fig. 4. The Shift in Temperature of Glass Transition Temperature (T_g) and Melting (Tm) of PVA and PVA-*Aloe vera* Nanofiber Membrane with Several Relative Humidity.

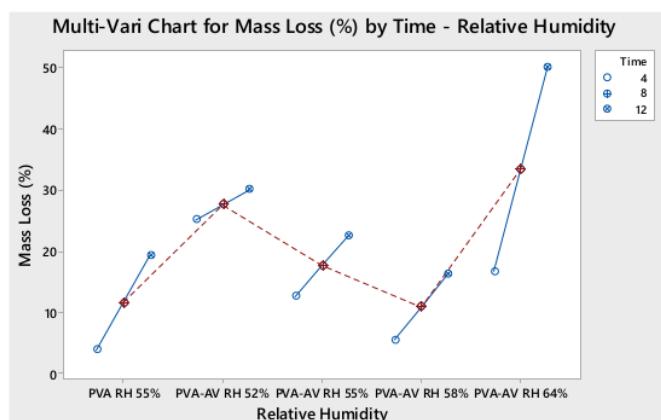


Fig. 5. Multi-Vari Chart of Mass Loss of PVA-*Aloe vera* Nanofiber Membrane due to Degradation with Several Relative Humidity Value Over Time.

Degradation Test. The degradation test on the PVA-*Aloe vera* samples showed that the sample degraded over time shown in Fig. 5. All the samples had a mass loss within only 12 minutes. They illustrated different behavior based on the relative humidity. The sample had lower mass loss with the increase of relative humidity. The sample with relative humidity of 52% had the mass loss between 25% and 30% while the sample with relative humidity of 58% had the mass loss from 5.4% to 16.2%. On the other hand, the mass loss increased drastically on the sample with relative humidity of 64% in a range of 16.6–50%. It indicated that the water content in the sample was too high for the sample to be maintained. Thus, the sample could be degraded easier than the other sample with lower relative humidity. Besides that, the changeover that in that sample was fast with 16.6% at 4 minutes and then it increased to 50% at 12 minutes. This result was significantly difference in term of relative humidity with p-value of 0.042 ($p < 0.05$) [7, 14].

Summary

The electrospun PVA-*Aloe vera* nanofiber membrane with several relative humidity values were synthesized. The FTIR spectra showed that the membrane already contained *Aloe vera*. The morphology test indicated that the fiber diameter of the sample increased with the addition of *Aloe vera* and decreased with the increase of relative humidity. The thermograms from DSC test showed that the temperature of glass transition (T_g) rose with higher relative humidity while the temperature of melting (Tm) declined in that condition. The degradation test indicated that the sample degraded overtime slower at relative humidity between 52% and 58% and then increased drastically at 64% relative humidity.

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