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Growth of collagen-nanosilver (Co-AgNP) biocomposite film with electrospinning method for wound healing applications

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Abstract. Silver nanoparticles (AgNP) are classified as metal-based nanoparticles and have received considerable attention among researchers in the application of wound healing, due to their physicochemical and biological properties. In this research silver nanoparticles (AgNP) will be combined with collagen to collagen-nanosilver (Co-AgNP) grown by electrospinning method into nanofiber film. The optimum parameter of the electrospinning process using an electric voltage of 25 kV at a spray distance of 20 cm becomes based on the process growth of Co-AgNP film. By using PVA as an electrospun polymer, the collagen-nanosilver composite (Co-AgNP) can be grown into a film that can be used as a wound dressing.

1. Introduction

Nanotechnology is a multidisciplinary field of scientific study that has attracted worldwide attention in various studies in science and industry. Nanotechnology can be applied to a variety of potential applications in medical science and biology, including medical diagnosis, medicine, biosensing, health care, drug delivery, coatings, medical devices, wound healing, food industry, cosmetics and environmental remediation (water purification) [1-5]. Nanoparticle-based therapies have various applications in wound care and healing. There are 2 (two) things related to the application of nanoparticles in wound healing, namely, nanomaterial used to heal wounds directly or nanomaterial acts as a carrier molecule to send pharmaceutically active compounds to the wound site [6].

Silver is a well-known metal and because of its unique properties is used for medicinal purposes. Silver is a soft, white, sparkling metal that has high thermal and electrical conductivity. Silver nanoparticles (AgNP) are classified as metal-based nanoparticles and have received considerable attention among researchers in the application of wound healing, due to their physicochemical and biological properties. The therapeutic benefits of silver have been known for years and have been used all the time [7,8]. Topical creams based on silver nanoparticles have been shown to be effective as antimicrobials to treat pathological infections in wound healing [7]. The development of new biocomposite materials with AgNP aims to achieve better treatment strategies related to wound healing with effective antimicrobial activity. Biopolymers are widely available from natural sources and are used in various applications in pharmacy, science, and medicine. Silver nanoparticles and biopolymer-based biomaterials (AgNP-BM) are non-cytotoxic and safe for patients in wound care management. The unique nature of AgNP-BM is effective wound healing in controlling the growth of microorganisms at the wound site and this strategy plays an important role in the treatment of acute and chronic wounds. Silver nanoparticles and biopolymer-based biomaterials offer a high degree of biocompatibility and



biodegradability in physiological conditions and can be considered as an effective material for wound dressing in wound care.

To ensure that the healing process takes place effectively, it is necessary to have a wound bandage that has been proven as the most sophisticated technology for treating many skin wounds due to thermal or physical damage [1]. Wound dressings must have many criteria: maintaining a moist wound environment, providing mechanical protection, promoting gas exchange, allowing removal without pain or trauma, being non-toxic and effective antibacterial, and accelerating wound healing [2]. Among these properties, antimicrobial properties are the most important factors for the healing process. Even when the wound is covered by a dressing, bacterial infection can still occur due to the moist environment trapped in it which provides space and nutrients for growing microorganisms [3]. Because of this, many disinfecting agents have been introduced for wound dressing such as chitosan, curcumin or silver nanoparticles (AgNP) [4]. Among them, using AgNP is the most sophisticated technique because of its proven high efficiency. Unlike other antibacterial agents, small silver ions released from AgNP that carry positive charges are easily drawn by more negative bacterial cell membranes. They trap, penetrate into the bacteria, disrupt the process of breathing and cause bacterial death [5]. Therefore, AgNP has antibacterial properties against various pathogens [6].

At present, many reports have focused on making and modifying scaffolds that are more likely to act as wound dressings [7-9]. Among biocompatible substitutes, poly- ϵ -caprolactone (PCL) is a special candidate thanks to its mechanical and structural properties [10]. Especially, PCL combined with the electrospinning method is expected to have synergistic benefits in wound care applications. In particular, electrospun nanofibers have been reported to have potential characteristics for wound dressings such as high porosity of nanofiber membranes that contribute effectively to gas exchange, providing the oxygen needed for cell respiration and fluid absorption [11]. In addition, electrospun PCL fiber bandages have superior mechanical and physical properties as well as elasticity which makes it flexible and functions as a protector against the environment. The electrospinning method can be used to coat drugs and other active substances into electrospun fibers [12]. Despite the many benefits of the application, PCL-based bandages that are attached to the electrospinning method also have drawbacks that affect the wound healing process. In particular, the porous surface structure of the electrospun bandage has a tendency to stick strongly to the wound surface because it absorbs moisture and empty pores thus providing an easy environment for infiltration and cell migration into the membrane, resulting in damage to the newly formed tissue when the dressing is removed and delays the process wound healing.

To prevent damage from releasing wound dressing, surface modification of electrospun has been investigated by combining with other biomaterials to utilize individual components of the wound dressing. The coating technique is more beneficial in terms of improving the active surface of the bandage while the mechanical and physical properties of the electrospun fiber are not disturbed. A wide range of polymers has been used as a coating layer [13]. Among them, agar has attracted attention due to its biocompatibility and ability to form hydrogels [14]. For wound dressing applications, gelatin is one of the main components that provide elasticity to connective tissue [15]. Another advantage of using gelatin as a stabilizer for AgNP is that it prevents AgNP aggregation and enhances the antibacterial effect of the pads. The use of composite pads that combine more than one ingredient to take advantage of characteristics that benefit them in repairing damaged skin tissue has gained interest recently.

Collagen is considered the easiest protein available. This protein is key in the extracellular matrix, consisting of one-third of the total amount of protein from the human body by weight [16]. The benefits of using collagen dressings in wound care are vast, including increasing the production of fibroblasts, the bioavailability of fibronectin, leukocytes, macrophages, fibroblasts and epithelial cells, and ultimately maintaining chemicals and thermostatic microenvironment to accelerate the healing process. Collagen-based pads are suitable for various types of wounds, including chronic wounds [17]. Collagen-based AgNP pads are good candidates for improving wound repair mechanisms with high efficiency of antimicrobial activity. Apart from antimicrobial activity, the function of AgNP present in collagen composites is primarily responsible for regulating collagen deposition and leads to increased alignment of fibrils in the wound healing process [18].

Electrospinning is a spinning technique using the electrostatic force approach to produce fiber from a polymer solution. The fiber produced from this method has a diameter range from nano to micrometer and a wider surface area than conventional spinning methods. This method is in great demand because it can control the production, fiber structure, porosity, orientation and dimensions of the resulting fiber. In this research, a collagen-nanosilver (Co-AgNP) nanofiber structure will be developed with an electrospinning method that can be used for dressing or wound dressing.

2. Experimental

The main materials and tools used in this study were electrospinning set up and test materials. The electrospinning set up materials include high voltage source (0-30KV), syringe pump 2 system with maximum size of 60 ml, system collector, plate / static conductor (20 cm x 35 cm), drum collector (0-3000 rpm) (L = 30 cm, d = 17 cm), nozzle system. Electrospinning test materials include polyvinyl alcohol, collagen, silver nitrate for nanosilver and DI water.

The electrospinning system generally contains a spinneret (a type of hypodermic syringe needle) connected to a high voltage of 0 to 30 kV for alternating current sources, syringe pumps and grounded collectors. The electrospinning set up is shown in Figure 1.

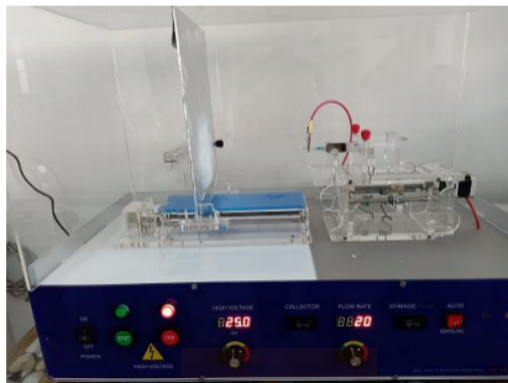


Figure 1 The electrospinning set up

To make the material that will be used as a source in the electrospinning method, PVA is chosen as a base material that can be mixed with the main material. PVA solution made of 2 grams of PVA powder plus aquades to a volume of 15 mL at a temperature of 80 °C and homogenized using a magnetic stirrer for 3 hours. The collagen solution was 1 gram of collagen crystal, each added with distilled water containing 0.5 M acetic acid to a volume of 10 mL was stirred with a magnetic stirrer for 2 hours. Then, PVA and collagen solutions are stirred for 3 hours. In the same method, the PVA solution is mixed with 10 drops of nanosilver to get the PVA-AgNP solution. Synthesis to produce a PVA-collagen-AgNP solution was carried out with the same composition, namely 2 grams of PVA plus 1 gram of collagen and 10 drops of nanosilver in a total time of 8 hours.

The bond characterization that occurs in PVA nanofibers is performed by Fourier Transform Infrared (FTIR, Digilab FTS7000) analysis so that the functional groups contained therein are known. UV-Visible spectrophotometer device was used to measure the absorption spectra of PVA nanofibers. The surface morphology of samples can be determined by photomicrograph and scanning electron microscope (SEM).

3. Results and Discussion

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FTIR characterization was carried out to determine the functional groups in the formed nanofiber. Absorption at a certain wavelength describes the existence of a specific functional group. When a material is irradiated with infrared radiation, there will be an interaction in the form of absorption of energy by the atoms or molecules of the material. The absorption of infrared radiation energy causes an increase in the vibrational amplitude of atoms in a molecule. From figure 2 we can see FTIR spectra of PVA nanofiber at the different concentrations of PVA with an absorption area of $2900-3000\text{ cm}^{-1}$ showing the C-H stretching group and its peak was detected at wave number 2939.29 cm^{-1} . There was another C-H stretching group at $3600-3800\text{ cm}^{-1}$ and the peak was detected at 3784.77 cm^{-1} . In addition, O-H stretching was found in the range of 3344.06 cm^{-1} .

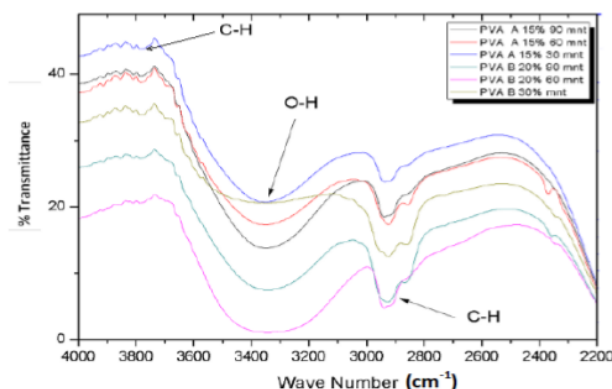


Figure 2. FTIR spectra of PVA nanofiber in various concentrations.

The UV-Vis absorbance spectrum of PVA nanofiber 15 wt% is shown in Figure 3. The optical absorbance against the wavelength in the wavelength range (200-500) nm. The absorbance spectrum shows a sharp absorption at a wavelength close to the edge of the absorption wavelength threshold. The peak of absorbance was observed at a wavelength of 285 nm corresponding to the characteristic absorption peak of PVA nanofiber [19].

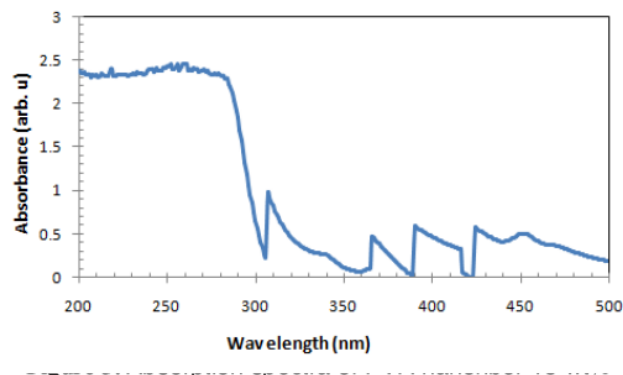


Figure 3. The optical absorbance against the wavelength in the wavelength range (200-500) nm

Figures 4 (a) and (b) show photomicrograph images of nanofiber PVA produced respectively from the electrospinning process of 15 and 20 wt% PVA solutions in a water solvent using an electric voltage

of 25 kV at a spray distance of 20 cm. There is no significant difference in the size of the fiber produced between the two samples. Therefore in this research, the electrospinning process has succeeded in producing the fiber size in a nanometer that is continuous and this is a consequence of the consistent flow of polymers that occur.

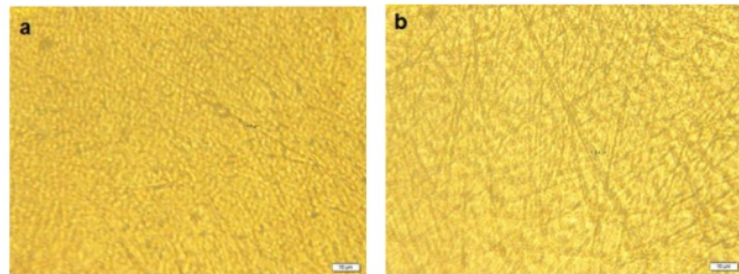


Figure 4. Photomicrograph images of PVA nanofiber with a)15 and b)20 wt%.

Figure 5 shows the film produced from the growth of PVA-nanosilver, PVA-collagen, and PVA-collagen-nanosilver (Co-AgNP). Nanofiber produced from the growth of this film is not visible because the resulting film is thick enough so that there is a build-up of nanofiber morphology.

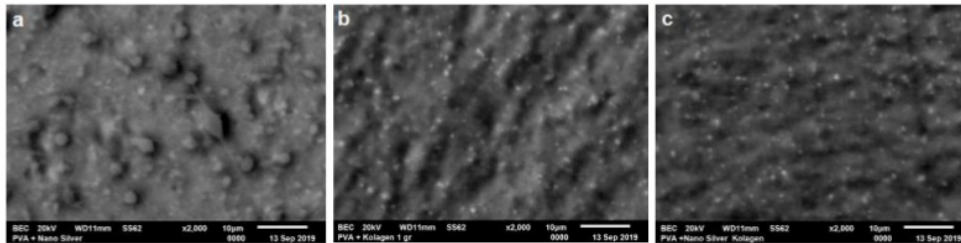


Figure 5. SEM images of a) PVA-nanosilver, b) PVA-collagen and c) PVA-collagen-nanosilver (Co-AgNP) films

Figure 6 shows the PVA-nanosilver, PVA-collagen, and PVA-collagen-nanosilver (Co-AgNP) films. Specifically for Co-AgNP films, it is predicted to be used as a wound dressing in which there are two ingredients, each of which has a function, namely collagen which helps the wound healing process and nanosilver as an anti-bacterial to prevent infection in the wound.



Figure 6. a) PVA-nanosilver film, b) PVA-collagen film and c) PVA-collagen-nanosilver (Co-AgNP) film.

3 4. Conclusions

The preparation of the composite nanofibers of PVA, PVA-nanosilver, PVA-collagen, and PVA-collagen-nanosilver (Co-AgNP) was studied. The electrospinning process of 15 and 20 wt% PVA solutions in a water solvent using an electric voltage of 25 kV at a spray distance of 20 cm become based to process growth of Co-AgNP film. The composite nanofibers of Co-AgNP could be successfully produced by injection of the treated mixture into the electrospinning machine under the optimum conditions.

Acknowledgments

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