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PHYSICAL EVALUATION OF PCL-AGNPS BIOCOMPOSITES AS GUIDED TISSUE REGENERATION MEMBRANE

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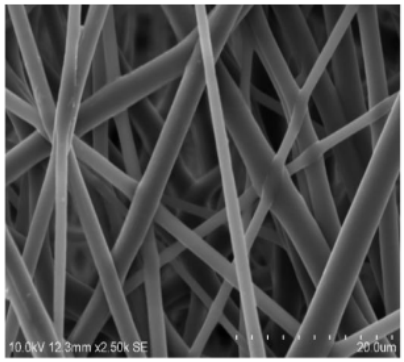
Graphical abstract



Abstract

Dental and mouth problems in Indonesia during 2007 to 2013 reportedly increased from 23.2% to 25.9% with the projection 65.275 million people with cases of periodontitis reaching 42.8%. One of clinical treatment of periodontitis is by using Guided Tissue Regeneration (GTR) method. This research was conducted to synthesize GTR membrane from Polycaprolactone (PCL) which is composed with AgNPs of Aloe vera biosynthesis product using electrospinning instrument. GTR membranes were synthesized by forming ratio of acetone and AgNPs which was obtained by mixing 30% Aloe vera extract with $AgNO_3$ solution where AgNPs had been characterized previously using XRD and PSA. In this study the comparison of solvents done as 100/0 (control), 90/10, 80/20, and 70/30 (v/v). Obtained GTR membranes are characterized by Scanning Electron Microscope and tensile strength characterization. From the analysis results known the best composition of PCL-AgNPs biocomposite as GTR membrane was found on the variable with ratio 70/30 (v/v) with fiber size 111,6 ± 22 nm, UTS value equal to 4,37 MPa with elongation equal to 204%. Based on the results of characterization, it showed that biocomposites PCL-AgNPs Aloe vera biosynthesis products have good potential as a guided tissue regeneration membrane.

Keywords: Guided tissue regeneration, polycaprolactone, AgNPs, electrospinning

1.0 INTRODUCTION

Lifestyle such as smoking, low consumption of antioxidant intake, poor nutritional intake such as high sugar consumption, and high levels of stress can increase the microbes associated with periodontal disease [1]. The most common periodontal disease seen in society is periodontitis. Periodontitis is a disease that causes chronic inflammation due to colonization of bacteria that can produce tooth and gingival damage, loss of connective tissue, erosion of alveolar bone, and tooth loss [2]. Tooth and mouth problems in Indonesia reported through InfoDATIN from 2007 to 2013 increased from 23.2% to 25.9%, which means that every four people have one person with dental and mouth problems, so the projection is estimated to be 65.275 million people with problems teeth and mouth. Periodontitis ranks second in dental and mouth disease which reaches 42.8% of Indonesian population [3].

One of the clinical treatments for periodontitis is the Guided Tissue Regeneration (GTR) method. GTR prevents epithelial tissue from spreading and ensures the growth of periodontal ligament cells in periodontal defects [4]. The currently used clinical GTR membrane is Polytetrafluoroethylene (PTFE). However, PTFE requires a second surgical procedure for removal of the membrane because it cannot be degraded. Failure also often occurs due to an infection caused by pathogen colonization at the wound site or a foreign body response. It urges the need for antibacterial biomaterials [5]. Poly (caprolactone) (PCL) in some journals can increase mechanical strength, have adjustable porosity of material and morphology, and facilitate the colonization and development of endothelial cells when their porosity has a micrometer scale [6, 7].

The addition of AgNPs provides more advantages in reducing pathogen colonization. AgNPs generally have stable chemical properties, antibacterial ability against both positive and negative gram and Multi Drug-Resistance (MDR) bacteria [8]. AgNPs are also reported to kill bacteria that cause periodontitis such as *Porphyromonas gingivalis, Treponema denticola,* and Staphylococcus aureus [9]. Based on the background, ther is necessity of Biocomposite PCL-AgNPs Biosynthesis Products Aloe vera As Membrane Guided Tissue Regeneration as solution for Periodontal Disorder Correction Therapy. In this study, the membrane will be characterized using Particle Size Analysis, XRD characterization, morphological characterization, and tensile strength characterization.

2.0 METHODOLOGY

All materials used are analytical grade materials. Acetone (98%) obtained from SAP Chemicals, Silver Nitrate ($AgNO_3$) obtained from Merck Millipore, Polycaprolactone (Mw 80.000) was obtained from Sigma Aldrich, *Aloe vera* species was *Aloe barbadensis miller*.

2.1 Synthesis of Silver Nanoparticles (AgNPs)

Aloe vera extract is obtained by cleaning and cutting *Aloe barbadensis Mille*r into small pieces. Furthermore, as many as 30grams of *Aloe vera* were put into 100 mL DI water at 80 °C for 15 minutes then filtered using Whatman Paper Filter no.1. The extracts of *Aloe vera* obtained are stored in dark glass bottles and low temperatures. Preparation of $AgNO_3$ solution was done by dissolving $AgNO_3$ powder as much as 1.575 gram in 1000 mL DI water. Both solutions are mixed with the ratio of Aloe / $AgNO_3$ as much as 1/9 (v/v), then the glass bottle is covered using aluminum foil to avoid the reduction of light and stored in room temperature. Store for 48 hours so the color of the solution changes from colorless to brownish.

2.2 Synthesis of Guided Tissue Regeneration Membranes

Synthesis of biocomposite was done using a set of electrospinning instruments. The solution for electrospinning was obtained by mixing AgNPs with acetone to form 10% solution concentration of AgNPs-Ace considering AgNPs was water-based. Firstly, AgNPs-Ace mixed with PCL solvent (acetone) with ratio of acetone/AgNPs-Ace are 100/0, 90/10, 80/20, and 70/30 (v/v) then add 10 g of PCL to form 10 wt% solution concentration of PCL/AgNPs biocomposite. PCL was dissolved in temperature of 50 ° C for 20 min to ensure the solution is well mixed. After the solution is formed, put it into a 10 mL syringe with 1 mm diameter of syringe tip. 5 mL of solution was processed with same flow rate so it is expected that the membrane has uniform thickness at each concentration. The electrospinning instrument uses a high voltage of 10-30 kV and distance of collector which has been covered by aluminium foil with syringe tip was 14 to 23 cm.

2.3 XRD and PSA Characterization

X-Rays Diffraction (XRD) characterization was performed using Cu-Ka radiation with a wavelength of 1,5406 Å, a voltage of 40 kV, and the scan speed is 0.02 °/min. Powder sample was used in XRD characterization. The powder was obtained by drying the AgNPs solution using an oven at 40 °C for 24 hours. The pattern generated by the XRD instrument is used to ensure whether the AgNPs have been formed. AgNPs solution then observed using Particle Size Analysis (PSA) instrument. PSA characterization aims to determine the size of particles, and distribution of AgNPs obtained.

2.4 Morphological Characterization

Morphological characterization was performed to determine the fiber obtained through electrospinning method on PCL-AgNPs biocomposite. Morphological characterization was performed on each sample with a Scanning Electron Microscope (SEM) instrument with 2500x and 10,000x magnifications. The sample was tested with 10 kV acceleration voltage and 5 µA current. The images then analyzed using ImageJ to determine fiber size and fiber orientation.

2.5 Tensile Strength Characterization

Tensile strength characterization was performed to determine mechanical properties of guided tissue regeneration membranes obtained. Membranes were cut with a width of 5 mm and a length of 16 mm refer to Carter (2016) methods. Sample thickness was calculated using benchtop thickness measurement instrument. Obtained sample was placed in the center of the paper holder used as a tensile strength characterization with a 6 mm gauge

length (Lg) and glued. Sample preparation is illustrated in Figure 1.

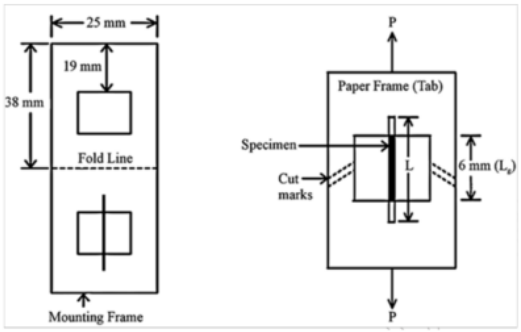


Figure 1 Sample preparation using paper holder (Caerter, Rahman dan Bhattari, 2016) [4]

Tensile strength characterization was carried out using a velocity Vi=10 mm/min and acquisition time data was 100 ms. Characterization was performed using 50 N load cell on instrument. When the sample has been properly installed, the paper holder was cut off according to the mark to reduce the tension caused by the paper at the start of the test.

3.0 RESULTS AND DISCUSSION

Preparation of AgNPs was succeed by adding Aloe vera extract to $AgNO_3$ solution. Colorless $AgNO_3$ solution turned to pale yellow when mixed with Aloe vera extract and turned to dark brown after 48 hours. The color change in solution occurs due to the reduction of silver ions (Ag+) become silver nanoparticles (AgNPs), refer to Chikdu (2015) the color of solution will darken as the incubation time gets longer. XRD characterization showed that the powder has (111) crystal lattice at 38.03° and (200) crystal lattice at 44.10° which are character of AgNPs lattice. It is known through Match!3 program that AgNPs have a face center cubic (FCC) crystal structure. The presence of (111) crystal lattice is the main lattice that contributes to the strong antibacterial properties besides particle size [10]. The presence of multiple peaks in the analysis results from the macromolecules of the reducing solution (Aloe vera extract). Extracts from plants are known to cause peaks in XRD results due to the presence of bio-organic substances [11].

It is known from the analysis data shown in Figure 2 that there is still an $AgNO_3$ phase even though the color of the solution has changed to dark brown. The existence of the $AgNO_3$ phase states that AgNPs have not been fully reduced. An alternative way to obtain more AgNPs phase can be done by increasing the volume of Aloe vera extract by increasing the reduction area of silver and the amount of AgNPs formation [12]. The presence of $AgNO_3$ phases are expressed as impurities. According to Qin (2016) and Korani (2011), the AgNPs and $AgNO_3$ phases do not have a significant effect on the cell but cell damage depends on the concentration of silver [13, 14].

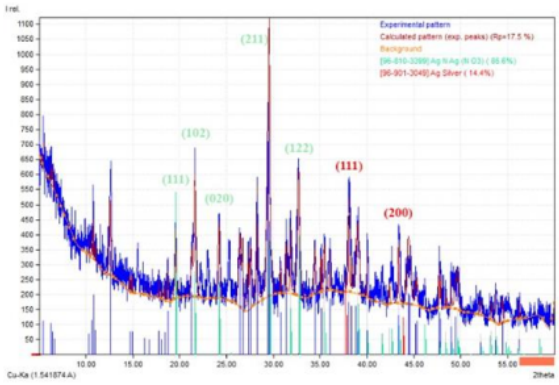


Figure 2 XRD pattern of AgNPs using Match!3

PSA Characterization Results

The result of PSA characterization shows that 24,1% AgNPs formed at 43,82 nm which shown in Figure 3. From the analysis data also known the existence of agglomerated particle which has size of 122 - 458 nm with very small percentage that is less than 0.7%. Molecules such as enzymes and proteins contained in Aloe vera extract affect the reduction process through weak bonds so as direct the growth of isotropic and spherical particles [10]. Weak bonds in this case are hydrogen bonds and / or electrostatic interactions that cause plant extracts to be a reducing agents and capping agents for silver nanoparticles [15]. The presence of agglomerated particles is caused by the weakness of the capping agent so that the particles can coalesce to form a more stable bond [15].

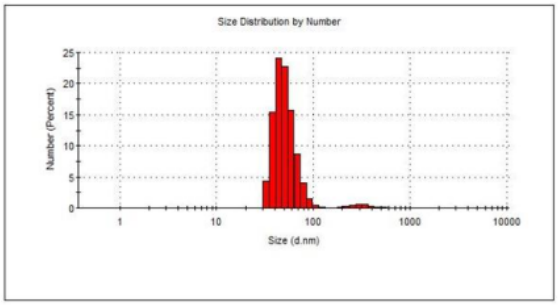


Figure 3 Particle size distribution of AgNPs

AgNPs are known to have more effective abilities because of its larger surface area per unit of weight [16]. Large or small particle size can occur due to the amount of composition of reducing chemical

compounds found in plants that vary greatly depending on species, climate, and growing conditions [17].

Guided Tissue Regeneration Membrane Synthesis and Morphological Characterization Results

The synthesis of PCL-AgNPs biocomposite was performed by mixing acetone solution with nanosilver first with ratio of 100/0, 90/10, 80/20, and 70/30 (ml/ml). Then, a PCL polymer with a concentration of 10 wt% was formed using a temperature of 50 °C while mixing for 20 minutes. Please note that the nanosilver formed in this study is water based so that nanosilver must be first dispersed in acetone to form AgNPs with a concentration of 10%. This dispersion treatment causes the dark brown color of AgNPs change to light brown. Clotting will occur when PCL is directly mixed with AgNPs without dispersion because PCL is a hydrophobic polymer. The PCL solution that acts as a control is colorless or clear Meanwhile, the color of the PCL-AgNPs biocomposite solution becomes orange-brown color indicate the higher volume of AgNPs, which shown in Figure 4.

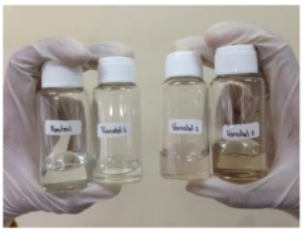
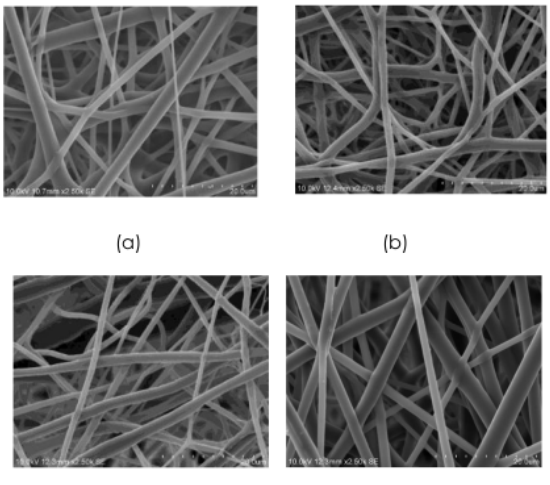


Figure 4 PCL-AgNPs solution

There were applied voltage difference when synthesizing GTR membrane. The GTR control membrane was formed at 21 kV, whereas the GTR PCL-AgNPs biocomposite membranes with ratio 90/10, 80/20, and 70/30 were formed at 18 kV, 16 kV, and 14 kV, respectively. Obtained membranes then observed using a light microscope known that a good fiber is without beads and there are no color differences in GTR membranes which are white. The application of different voltages during the GTR membrane formation process is due to the difference in the number of AgNPs in the forming solution, when AgNPs are increase so that the electrical charge and the conductivity of the solution are increase too [18]. Please note that if the voltage used is uniform causes GTR membranes formed through electrospray process. The electrospray membrane is undesirable in this study because fiber was not obtained in membranes, electrospray tend to form nano-sized particle membrane. Electrospray membranes known has poor tensile strength and does not meet GTR membrane requirement. GTR membrane is important to support the growth of an extracellular matrix consisting of fibers with diameter of 50-500 nm in cell development [19].

Morphological characterization results using Scanning Electron Microscope (SEM) indicates that obtained membrane has fibers with a smooth surface, homogeneous cylindrical shape, no pores on the fibers, and no grains or beads. In addition, it is known that in all surface area there are no fibers with micro-ribbon characteristics which can caused by high viscosity of solution [20]. The properties of solvents such as conductivity and boiling point also have an important influence in the formation of fiber and morphology. Acetone considered as a good solvent for PCL because its tend to evaporate fast and does not makes pores in fiber during the electrospinning process [21].

The results of morphological characterization using SEM were then analyzed using ImageJ with the DiameterJ plug-in which can calculate the orientation of fiber and fiber diameter. It is known through the analysis that the GTR control membrane has a fiber size of 113.3 ± 18.5 nm; Whereas, PCL-AgNPs Biocomposite GTR membrane with ratio 90/10, 80/20, and 70/30 respectively are 108,6 ± 26 nm; 106.9 ± 28.2 nm; and 111.6 ± 22 nm which can be seen in Figure 5. GTR membranes showed a good results considering the diameter of the membrane fibers can resemble the scale and morphology of extracellular matrix which has a size of 50 - 500 nm [19].



(a) (b)

(c) (d)

Figure 5 Morphological data of: PCL GTR membrane (a); 90/10 PCL AgNPs GTR membrane (b); AgNPs GTR membrane (c); 70/30 PCL AgNPs GTR membrane (d)

Theoretically, the addition of AgNPs in the solution increases the electrical charge and conductivity resulting in a smaller diameter as the number of AgNPs increases. The transfer of charge from the input or supply voltage to the polymer in the solution is related to the conductivity, when the higher

conductivity, the more ions are formed which cause the repulsion and form the smaller fibers [22]. However, it should be noted that the formation of the fibers is also controlled by applied voltage, when the applied voltage consider is suitable, increasing the amount of AgNPs in the solution increases the whipping action to form a smaller fiber diameter [23]. In data shown, PCL-AgNPs membrane with ratio 70/30 is less harmonious with the theories, this can be caused by collector distance parameters are too close. The diameter of the fibers will be smaller when the electric field in the electrospinning instrument increases, but when the distance is not far enough then the whipping process will not proceed well resulting in large diameter even though the electric field is also high [24].

Tensile Strength Characterization Results

Mechanical characterization aimed to get Ultimate Tensile Strength (UTS) value by determining maximum tensile value of GTR membrane that can be tolerate before its broken. UTS become one of the indications of difficulty in applying membrane during the operation process. A GTR membranes require stable structure when it receives mechanical stress and flexible when applied. The value of UTS GTR membrane approaching periodontal tissue is preferred, this is to avoid the "stress-shielding" effect and maintain mechanical strength when cell growth occurs in vitro, in vivo, and also tissue modeling process [4]. The UTS mean value of GTR control membrane was 2.852 ± 0.4050 MPa, and the value of GTR biocomposite membranes with ratio 90/10, 80/20, and 70/30 were 3.901 ± 0.4646 MPa, 1.891 ± 0.0436 MPa, and 4,370 ± 0.8106 MPa which shown in Figure 6. Addition of AgNPs to the solution from experiment shown that the tensile strength of GTR membrane was increase. AgNPs in polymers tend to form aggregates and form chain-like structures when mixed well performed prior to electrospinning [25].

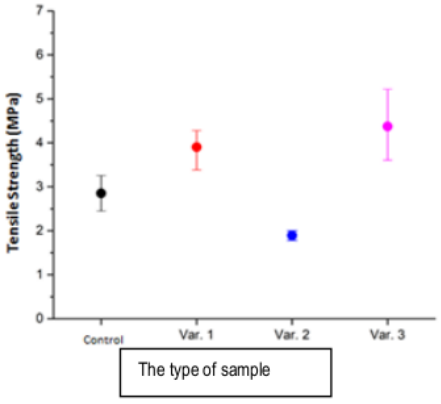
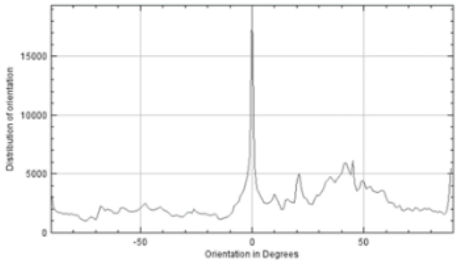


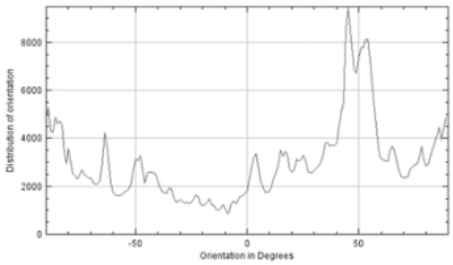
Figure 6 UTS value of GTR membranes

In this research also shown that the sample with composition AgNPs-Ace 80/20 has a small value, this phenomena are attributed to many reasons such as composition, size, fiber orientation, molecular structure, fiber arrangement, and post-processing conditions [25]. In a study conducted by Anindyati (2015) [26], structural damage may occur during the testing process so that the force value when it pulls becomes low. The structural damage itself may cause by repulsion and the fibers are not able to withstand the load or the stress are present in membranes [26].

Considering that orientation is one of the main parameters, the orientation of GTR membrane were analyzed through morphological characterization data using ImageJ. Analysis data shown that GTR PCL-AgNPs biocomposite membrane with ratio 80/20 has a diverse and random orientation compared with others, data shown in Figure 7. Random orientation caused stress from load cell become not same at all area of the membranes so that its tend to produce low UTS value. Random orientation itself can be affected by several factors. One of the factors is applied voltage when producing membranes through electrospinning process. Applied voltage causes break surface tension on solution obtained, if the applied voltage is too high it can cause fibers have small diameter but also increasing instability which can cause increased whipping movement of fibers so that can form random fiber orientation [23].



(a)



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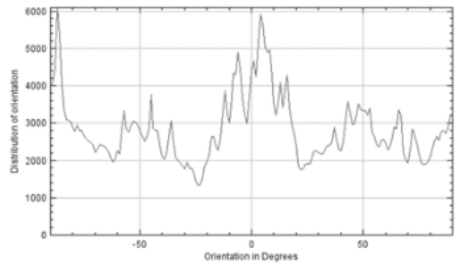
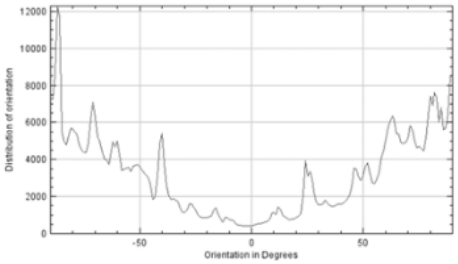


(c)



(d)

Figure 7 Fibers orientation of: PCL GTR membrane (a); 90/10 PCL AgNPs GTR membrane (b); 80/20 PCL AgNPs GTR membrane (c); 70/30 PCL AgNPs GTR membrane (d)

Overall, UTS value of the biocomposite GTR membrane increases with the addition of AgNPs compared to pure PCL, in addition to elongation of the higher biocomposites, this is due to the distribution of AgNPs in the polymer thereby minimizing the formation of centralized stresses on the membrane under test and increasing the surface area to deliver the stress from the matrix polymer to the filler [27]. In this study data were obtained in line with Anindyati's (2015) [26] study where random fiber orientation contributed to higher elongation values. The data show that the fiber orientation of the majority GTR control membrane at 0 ° has a smaller elongation value than the others [26]. From this research it is known that GTR biocomposite PCL-AgNPs membrane with 90/10 and 70/30 ratio have fulfilled the criteria of tensile strength as GTR membrane candidate having range of 3.5-22.5 MPa [19].

4.0 CONCLUSION

The effect of concentration variation through the amount of addition of AgNPs in PCL-AgNPs biocomposite manufacturing as GTR membrane of morphological characterization showed that the addition of AgNPs minimizes fiber diameter. While the tensile strength characterization results indicate the addition of AgNPs increases the value of Ultimate Tensile Strength (UTS), although UTS values are also influenced by fiber orientation.

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