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Biological Evaluation of PCL-AgNPs Biocomposites as Guided Tissue Regeneration Membranes

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Abstract: More than half of the world's population still experiences dental and mouth disease which causes disruption to the quality of human life. The number of untreated dental and oral diseases increased by 40% from 1990 to 2015 which reached 3.5 billion people and periodontal disease in severe conditions reached 573 million. One of the cases of periodontal abnormalities is the Guided tissue regeneration (GTR) method. In this study the formation of GTR membranes derived from biocomposite PCL-AgNPs using the electrospinning method. The results obtained through FTIR characterization, degradation, antibacterial, and cytotoxicity that the addition of AgNPs did not show changes in functional groups. All biocomposite GTR membrane PCL-AgNPs samples showed that AgNPs only physically bonded to PCL. Degradation characterization shows that the addition of AgNPs causes the rate of degradation to increase with increasing number of AgNPs. The addition of AgNPs also shows the ability of membranes to inhibit bacteria and viability decreases with increasing number of AgNPs.

INTRODUCTION

More than half of the world's population still experiences dental and mouth disease which can interfere the quality of human life. The number of untreated dental and oral diseases increased by 40% from 1990 to 2015 which reached 3.5 billion people and periodontal disease in severe conditions reached 573 million [1]. Periodontitis ranks second in dental and oral disease which reaches 42.8% of the Indonesian population. One of the clinical treatments for periodontitis is the Guided Tissue Regeneration or GTR method. The surgical procedure requires a retaining membrane or barrier called the GTR which functions to prevent the epithelial tissue from expelling and ensures the growth of periodontal ligament cells in the area of the periodontal defect. GTR membrane aims to prevent the outgoing epithelium and gingival tissue to grow in the area of the defect when the process of proliferation of the periodontal ligament so as to support the growth of cementum, periodontal ligament and alveolar bone [2]. Clinical results using e-PTFE membranes are often disappointing and procedures for removing the membrane from the periodontal defect area are necessary because they cannot be degraded. Besides this membrane that can not be degraded also raises higher costs, infection during surgery and after surgery, and tissue growth that is less than the maximum [3],[4]. Considering the infection occurs during surgery, it is possible that there are bacteria that cause the condition of Multidrug Resistance (MDR) bacteria, therefore we need a technique that can kill bacteria on the membrane surface effectively [4].

At present membranes that can be degraded and have antibacterial properties are being developed, but clinical results show that membranes have unpredictable results [2]. PCL can be degraded by bulk erosion by hydrolysis of polymer chains and then eliminated from the body through the citric acid cycle so that the rate of degradation can be predicted [5]. Nanosilver is one of the metals of great interest in molecular diagnostics, therapy and several medical procedures. The application of nanosilver as an antibacterial activity has the potential to provide solutions to

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problems in antibiotic resistant bacteria. Nanosilver synthesis using microorganisms or plant extracts such as Aloe vera has the advantage of being environmentally friendly, simple, low cost, and providing excellent results [6], [7]. Therefore in this study the addition of AgNPs through biosynthesis using Aloe vera on PCL to form a GTR membrane that can be degraded and has antibacterial properties for cases of periodontal abnormalities. In this study, Fourier Transform Infra-red (FTIR), Degradation, Antibacterial and Cytotoxicity Characterization were carried out.

MATERIALS AND METHODS

Materials

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) obtained from Sigma Aldrich, Aloe vera type Aloe barbadensis miller, Mueller Hinton Agar (CM0337) obtained from OXOID.

Synthesis

Synthesis of Silver Nanoparticles (AgNPs)

Aloe vera extract is obtained by cleaning and cutting Aloe barbadensis Miller with a small size. Next, as much as 30gram Aloe vera that has been cut is put into 100 mL DI water at 80 ° C for 15 minutes then filtered using Whatmann Paper Filter No. 1. Aloe vera extract obtained is stored in dark glass bottles and low temperature. Furthermore, a 1.575gram $AgNO_3$ solution was dissolved in 1000 mL DI water. Both solutions were mixed with a 1/9 (v / v) Aloe / $AgNO_3$ ratio then the container was closed using aluminum foil to avoid light reduction and stored at room temperature. Store for 48 hours so that the color of the solution changes from clear, colorless to brownish.

Synthesis of Guided Tissue Regeneration Membranes

Biocomposite synthesis is carried out using a set of electrospinning instruments. The solution for electrospinning was obtained by mixing AgNPs of Aloe vera biosynthetic products with acetone first to form a solution concentration of 10%, then the formed solution was mixed into the acetone solution as PCL solvent with a ratio of 100/0, 90/10, 80/20, 70 / 30 in a volume (ml) / volume (ml) ratio then PCL is added as much as 10gram to form a solution with a concentration of 10wt% using a temperature of 50 ° C for 20 minutes to ensure the solution is well mixed. After the solution is formed, then the solution is put into a syringe measuring 10 ml as much as 5 ml so that it is expected that the membrane has a uniform thickness at each concentration. The electrospinning instrument uses a high voltage of 10-30 kV with a static syringe tip that has a diameter of 1mm having a range of 16 to 23 cm to the collector tube coated by aluminum foil.

Characterization

Characterization of Fourier Transform Infra-Red (FTIR) and Morphological

FTIR characterization was carried out using FTIR instrumentation with absorption waves of 400 to 4000 cm^{-1} using solid samples which were added a little KBr powder [8]. The solid sample used is a GTR control membrane in the form of a pure PCL membrane.

Degradation Characterization

Degradation characterization is done using PBS to determine the rate of degradation by calculating the mass loss. This test is done by placing the sample in tubes filled with 10 ml PBS solution at 37 ° C for one day, three days, seven days and three weeks. The degradation rate is determined using the dry weight maintained by the formula: ((DMt / DMi) x 100%);DMt is the weight after the degradation test and DMi is the initial weight of the sample before the degradation test [9].

Antibacterial Characterization

Antibacterial characterization was carried out using Staphylococcus aureus as gram-positive bacteria and E.Coli as gram-negative bacteria with media *Mueller Hinton Agar* (CM0337) obtained from OXOIDusing a 9cm petri dish. Membrane samples are formed into round with a diameter of 5mm.

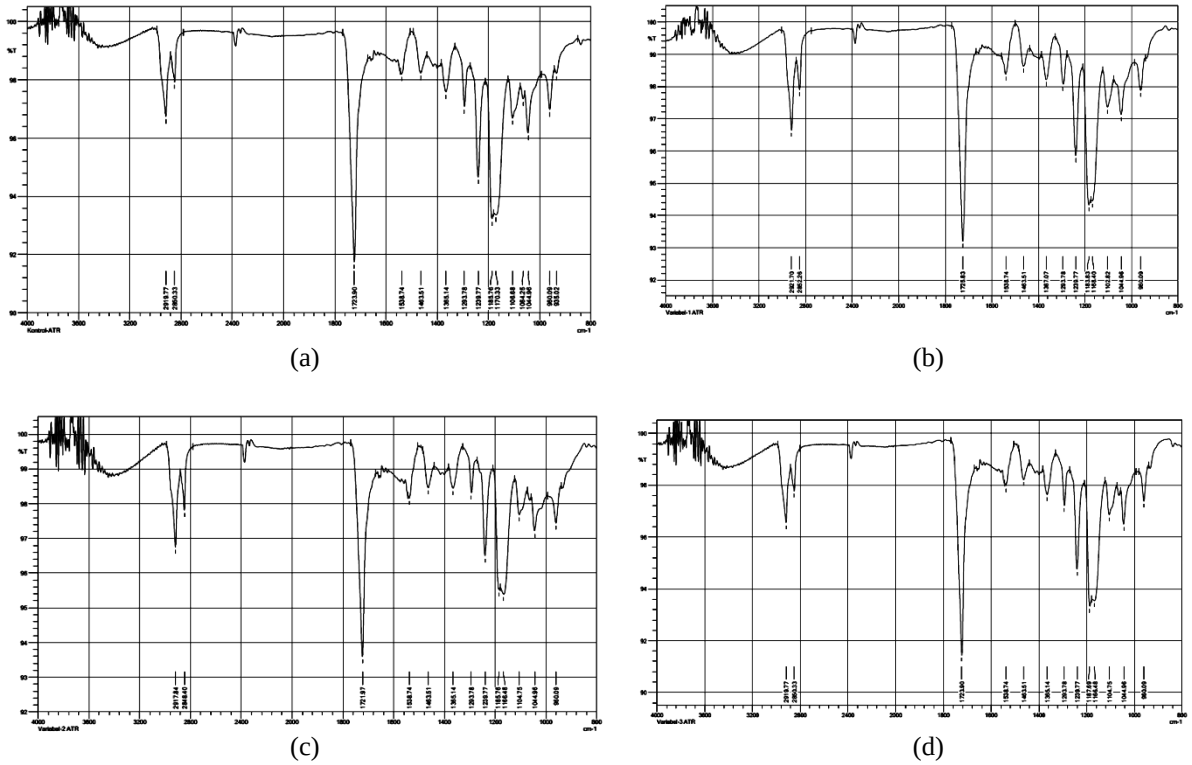
Cytotoxicity Characterization

Cytotoxicity characterization was carried out using BHK21 cells with DMEM media. Characterization was carried out on each sample using 5 repetitions in each sample. Then the plate that has been cultured for 24 hours and has been given a sample is calculated using Elisa Reader to determine the number of cells that are still alive.

RESULTS AND DISCUSSION

FTIR Characterization

FTIR characterization was carried out on membranes that had been formed and identified using the Shimadzu IRTracer-100 instrument with the Attenuated Total Reflectance (ATR) method where material verification and identification can be done accurately. The sample identified was 1 cm x 1 cm with the addition of KBR powder. The existence of this H_2O molecule is proven by the presence of noise in the 4000-3300 cm^{-1} wave absorption similar to the Sigma-Aldrich PCL product database.



(a) (b) (c) (d)

FIGURE 1. Results of FTIR Membrane GTR (a) Control, (b) PCL-AgNPs Var.1, (c) PCL-AgNPs Var. 2, and (d) PCL-AgNPs Var. 3.

In the results of the characterization note that there is a strain C=O with absorption waves around 1720 cm^{-1}, asymmetrical and symmetrical C-H_2 strain in absorption waves around 2920 cm^{-1} and 2850 cm^{-1}, CO and CC strain in absorption waves around 1290 cm^{-1}, and COC strain in regions of 1239 cm^{-1} and 1170 cm^{-1} which is a

characteristic of polycaprolactone. From the results of this characterization it is known that the addition of AgNPs does not change the chemical structure of the polymer or it can be said that AgNPs only bind physically. The amount of AgNPs concentration in the higher solution also does not change the functional groups contained in the polymer, this can be known through FIGURE 1. All the peaks contained in Figure also successfully analyzed by strain of the functional groups listed in TABLE 1.

TABLE 1. Wave Absorption and Analysis of PCL Membrane Function Groups (Controls) and PCL-AgNPs Biocomposite Membranes

Control	Variable 1	Variable2	Variable 3	Analysis
2919.77	2921.7	2917.84	2919.77	*CH_2 Asymetric Stretch*
2850.33	2852.26	2848.4	2850.33	*CH_2 Symetric Stretch*
1723.9	1725.83	1721.97	1723.9	*C=O Stretch*
1538.51	1538.74	1538.74	1538.74	*Water dimer*
1463.51	1463.51	1463.51	1463.51	*CH Scissoring and Symetric Deformation*
1365.14	1367.07	1365.14	1365.14	
1293.78	1293.78	1293.78	1293.78	*CO and CC Stretching in the Crystalline Phase*
1239.77	1239.77	1239.77	1239.77	*COC Asymetric Stretch*
1185.76	1183.83	1185.76	1187.69	*OCO Stretch*
1170.33	1168.4	1166.48	1166.48	*COC Symetric Stretch*
1106.68	1102.82	1104.75	1104.75	*CO Stretch Secondary Alcohol*
1064.25				*CH Rocking*
1044.96	1044.96	1044.96	1044.96	*CC Stretch Amorphous*
960.09	960.09	960.09	960.09	*COC Symetric Stretching*
935.02				*CC Rocking*

Degradation Characterization

The degradation characterization aims to determine the rate of degradation and prove that the PCL-AgNPs biocomposite GTR membrane can be degraded. This is an important parameter considering that the GTR membrane used clinically cannot be degraded, namely the PTFE membrane, thus requiring surgical removal. A good GTR membrane has degradation properties that are appropriate to the formation of new tissue, and physical characteristics so that it can be applied to the body, and the strength is enough to avoid damage to the membrane and perform functions as barrier [10]. GTR membranes must be present and function in the post-operative area for at least four to six weeks to enable successful regeneration of the periodontal system [11].

TABLE 2. Degradation Results of GTR Control and Biocomposite PCL-AgNPs for 21 Days

Variable	Day(s)			
	1	3	7	21
Control	0.0000 %	0.0000%	0.9009 %	3.3333 %
Var. 1	2.0833 %	4.5455%	5.3606 %	7.0085 %
Var. 2	2.7778 %	5.5944%	5.5944 %	8.0000 %
Var. 3	4.7619 %	6.3492%	6.3492 %	8.2332 %

Research data obtained from a 21-day trial showed that the control GTR membrane and the PCL-AgNPs biocomposite GTR membrane showed that the membrane could be degraded. From these data listed in Table 2, shown that Var 3 (ratio of 70/30) is GTR membrane fastest degraded, other than that the data shows that the increasing amount of AgNPs in the solution of the degradation rate also increases as listed in Figure 2. The increased rate of degradation in the GTR membrane that was formed was allegedly due to the increased hydrophilic nature of the membrane [12], [13].

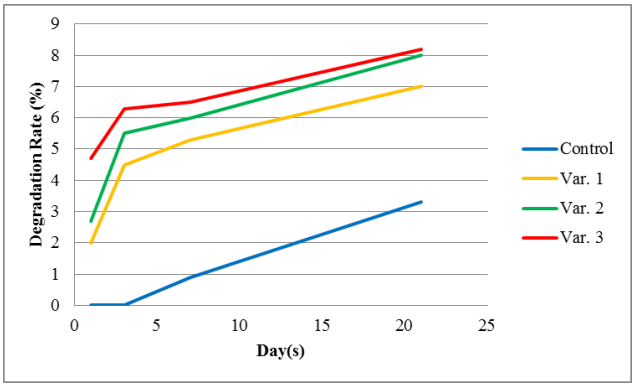


FIGURE 2. Degradation Chart of GTR Control and Biocomposite PCL-AgNPs for 21 Days

In the GTR membrane control is indicated the process of degradation that occurs through surface degradation so that the membrane is degraded very slowly. Surface degradation involves hydrolytic cleavage of the polymer main chain on the surface, this condition occurs when the rate of cutting of the hydrolytic chain and the oligopolymers and monomers that form diffuses around the main chain faster than the rate of water or PBS into the bulk polymer. In this process the molecular weight of the inner polymer is not affected so that the polymer only wears out over time for a long time [5]. In contrast to the PCL-AgNPs biocomposite GTR membrane, the degeneration process that occurs is mass degradation. Since the hydrophilic nature of the PCL-AgNPs biocomposite GTR membrane is increasing, it is presumed that water can penetrate the entire polymer bulk causing hydrolysis in all polymer networks. Cutting hydrolytic chains in this process occurs randomly which causes a reduction in molecular weight in all regions. Water or a solution that can diffuse into the bulk polymer hydrolyzes the chain making it possible to form monomers and oligopolymers that spread so that erosion will occur gradually until the equilibrium point is reached [13]. Considering that mass degradation occurred on PCL-AgNPs biocomposite GTR membranes, a greater percentage of degraded mass was obtained when compared to the control GTR membrane which was allegedly degraded on its surface.

Related to the increasing percentage of degraded mass with increasing number of AgNPs, it can be caused by the uneven distribution of AgNPs in all fibers and the ability of AgNPs to improve the hydrophilic nature of the membrane so that if the number of AgNPs is greater than the area or fibers with AgNPs have higher hydrophilic properties than those of AgNPs others cause the area to be degraded first by the mechanism bulk degradation random ones [13].

Antibacterial Characterization

Antibacterial characterization aims to determine the ability of membranes to inhibit bacteria at the periodontal defect site. Through the disc diffusion method it is known that biocomposite PCL-AgNPs can inhibit bacteria in the periodontal defect area. AgNPs can inhibit the growth of S. aureus bacteria with a diameter of 12 to 17 mm and E. coli bacteria with a diameter of 13 to 18 mm as shown in Figure 3. It is known that the more AgNPs in the GTR membrane, the greater the bacterial inhibitory area. GTR membrane that has been formed is stated to have a very good and effective inhibitory area against bacteria with gram positive or negative [14, 15].

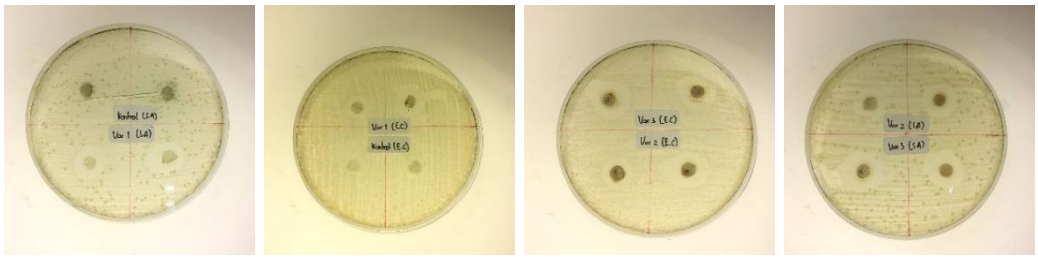


FIGURE 3. Graph of Bacterial Inhibition Zones

Cytotoxicity Characterization

Cytotoxicity characterization was added in this study to ensure that the guided tissue regeneration membrane obtained was safe when applied to the location of periodontal defects. Cytotoxicity characterization used was in vitro MTT Assay test using BHK21 cells. From the results of cytotoxicity test it is known that the increasing concentration of AgNPs in the membrane then the percentage of living cells will decrease as shown in Figure 4. However, the amount of AgNPS on the membrane can still be said to be safe considering the percentage of cell life above 60% which refers to ISO [16].

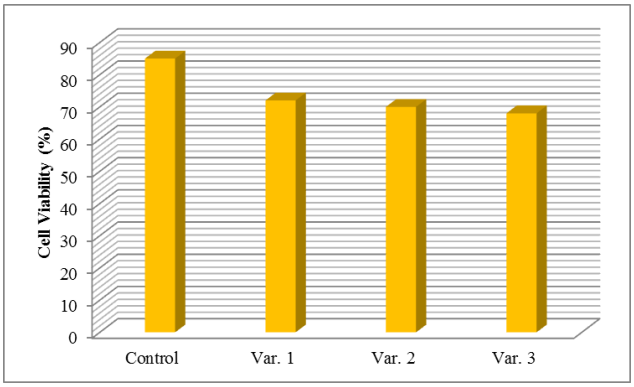


FIGURE 4. Results of Cytotoxicity Characterization

CONCLUSIONS

The addition of AgNPs did not show changes in functional groups and shows the ability of membranes to inhibit bacteria and viability decreases with increasing number of AgNPs. All biocomposite GTR membrane PCL-AgNPs samples showed that AgNPs only physically bonded to PCL. Degradation characterization shows that the addition of AgNPs causes the rate of degradation to increase with increasing number of AgNPs. The best composition of PCL-AgNPs biocomposite as GTR membrane was found in a variable with a ratio of 70/30 (v/v) with a fiber size of 111.6 ± 22 nm, a mass degraded for 21 days of 8.2332%, and the average value of Ultimate Tensile Strength (UTS) of 4.19 ± 0.840 MPa with an average elongation of 204%. In this study additional studies need to be carried out such as the ability of GTR to facilitate cell growth in vitro and in vivo.

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