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Synthesis and Characterization of Bacterial Cellulose – Collagen – Glycerol as Artificial Dura Mater

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The occurrence of head trauma could cause problems such as concussions. The occurrence of a collision sometimes could cause direct trauma that causes dural tear which if not promptly treated, it could be fatal. In the previous research, it has created the dura mater derived from bacterial cellulose - collagen, but the sample was too stiff and fragile. This study is performed addition of plasticizers with a variation of 0.1%, 0.2%, and 0.3%, to decrease the tensile strength. The test results of functional groups test using Fourier Transform Infrared showed that the test group had their functional groups of O-H stretching that provided information on the presence of glycerol in the samples of cellulose I and C-O bond stretching at 1053.85 cm^{-1} which is an group I amide and indicates the presence of collagen. The best sample is sample with the addition of glycerol 0.3% with a tensile strength of 23.58 MPa. This value is corresponded to the standard artificial dura mater which was in the range 0.6 to 16 MPa and elongation value at 8.6%, according to the artificial dura elongation of 7-14%. In the cytotoxicity test, it was obtained a percentage of living cells above 50%, which meant that the material was not toxic when applied to the body and the most optimal degradation test was on the sample of bacterial cellulose - collagen - 0.1% glycerol for 14 days which was degraded 18.85%. The conclusion of this study was that microbial cellulose-collagen-glycerol is a material that has potential as a candidate artificial dura mater.

Keywords: Artificial dura mater; bacterial cellulose; collagen; glycerol

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The occurrence of head trauma could cause a disturbance, such as concussion. In Indonesia, the concussion case is in the sixth position of death cause with 0.4% occurrence [1]. Besides that, the number of head trauma case treated in the hospital is the second biggest death cause after stroke with 4.37% [2].

The abnormalities caused by the head trauma could be an epidural hematoma, subdural hematoma, subarachnoid bleeding, and intracranial infection. Those abnormalities are related to 3 crucial components of brain layers, which are dura mater, arachnoid mater, and pia mater. The incident of sudden severe bump could cause direct trauma which leads to the tear of dura mater. When the dura mater is broken, then the brain as a vital organ would be disturbed and if it is not treated immediately, it would be fatal.

With that evidence, it is needed to have a dura mater substitute that is biocompatible so that it is safe to use [3]. In the previous study which was performed by Adanti et al at 2017 [4], an artificial dura mater has been synthesized originated from bacterial cellulose (BC) by using Acetobacter xylinum. However, the sample was still too stiff with elongation of 8.33% and tensile strength of 50.74 MPa, while the standard value of artificial dura meter tensile strength was 0.6 – 16 MPa [5]. Adanti research et al [4] was showed elongation value 8.33% in sample A (SB-Collagen 0.4%) compared to the control sample. While the elongation value tend to decrease subsequently due to the decline of collagen concentration (sample B with SB-Collagen 0.5%, sample C with SB-Collagen 0.6%, sample E with SB-Collagen 0.7%) which are 4.70%, 2.72% and 1.76%.

Thus, it is needed to add a plasticizer to control the tensile strength corresponding to the standard of artificial dura mater tensile strength. By that reason, the plasticizer would be added to, such as glycerol, with a variation of 0.1-0.3% m/v to analyze the possibility of the decrease of the tensile strength of the bacterial cellulose [6]. The bacterial cellulose is a natural polymer that is biodegradable and has high mechanical strength [7]. Besides that, this bacterial cellulose could be easily made from the coconut water.

The Fourier Transform Infrared (FTIR) test is performed to determine the functional group in the membrane, tensile strength test (Autograph) to

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determine the mechanical strength, the morphology test by using Scanning Electron Microscope (SEM) to determine the surface structure and sample pore size, the cytotoxicity by using MTT Assay and degradation test to determine the material degradation mass.

MATERIALS AND METHODS

Materials

The materials used in this study were Glycerol pro analysis 87% Merck, collagen from Kakap fish skin from National Nuclear Energy Agency (BATAN), South Jakarta, Indonesia.

Acetobacter xylinum used in this study was obtained from microbiology laboratory, faculty of science and technology, Universitas Airlangga.

Methods

The synthesis process was started with the making of bacteria medium from coconut water. 300 mL filtered coconut water, 1 gram urea, 30 grams glucose, 6 mL acetic acid and glycerol with concentration variation of 0.1%, 0.2%, and 0.3% and heated until boiling. Then, the bacterial media was sterilized by using an autoclave. After that, the media culture was placed in a container or fermented jar and set aside until cool down. The 10 mL *Acetobacter xylinum* starter was added in each variation and let aside for one week. After one week, the bacterial cellulose was repeatedly rinsed by using distilled water and dried by using free-dry method for 3 days [6, 8].

The making of collagen solution of 0.1 m/v % was started with dissolving 2 grams of citric acid in 100 ml of water and the 0.1 gram collagen was added to it gradually. When it was homogeneous, the bacterial cellulose membrane was immersed in this solution for 6 hours. This membrane was sterilized with alcohol and kept in the normal saline in the freezer [9-10].

RESULTS

Fourier Transform Infra Red Test (FTIR)

In this study conducted a few variations on the glycerol concentration is 0.1%, 0.2%, 0.3%. Test of functional groups using Fourier Transform Infrared (FTIR). (Figure 1).

The FTIR result is showed sharp peak which indicate –OH cluster in the range of wave number 3600-3100 cm^{-1} and amide C-O stretching cluster in the range of wave number 1075-1000 cm^{-1} which is showed collagen indication. This results are demonstrated that the synthesis is succeed.

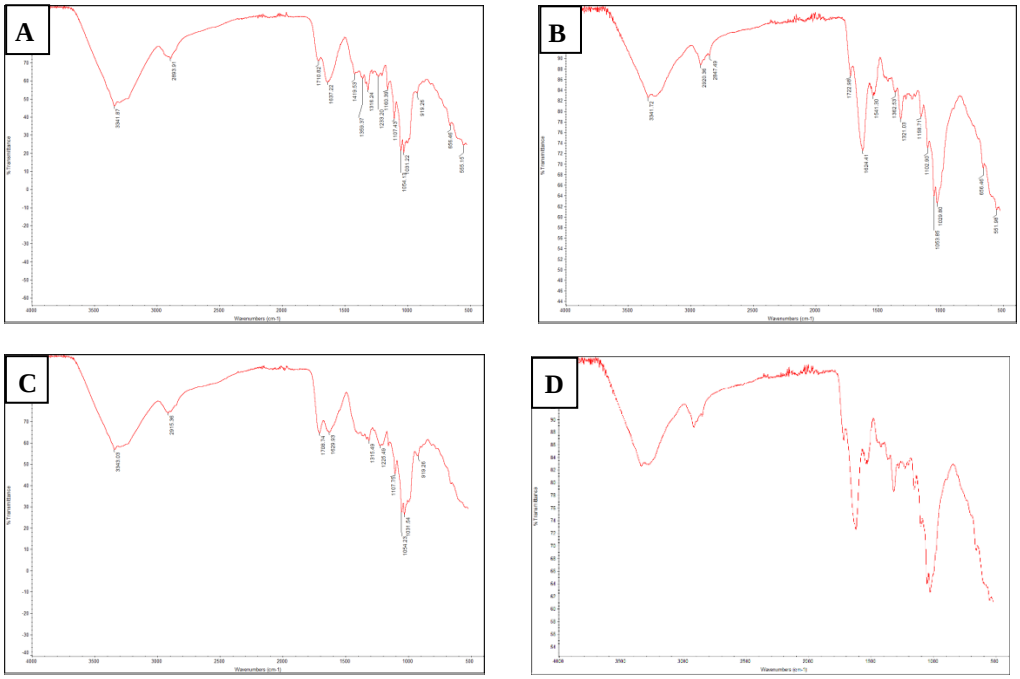

Figure 1: The result of functional group test (a) BC-Collagen (b) BC-Collagen-Glycerol 0.1% (c) BC-Collagen-Glycerol 0.2% (d) BC-Collagen-Glycerol 0.3%.

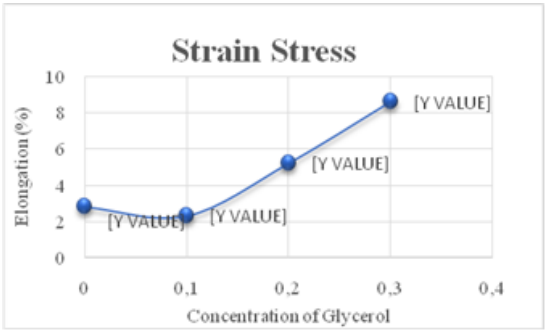

Figure 2: The Result of Tensile Strength of BC-Collagen-Glycerol with Glycerol Concentration Variation

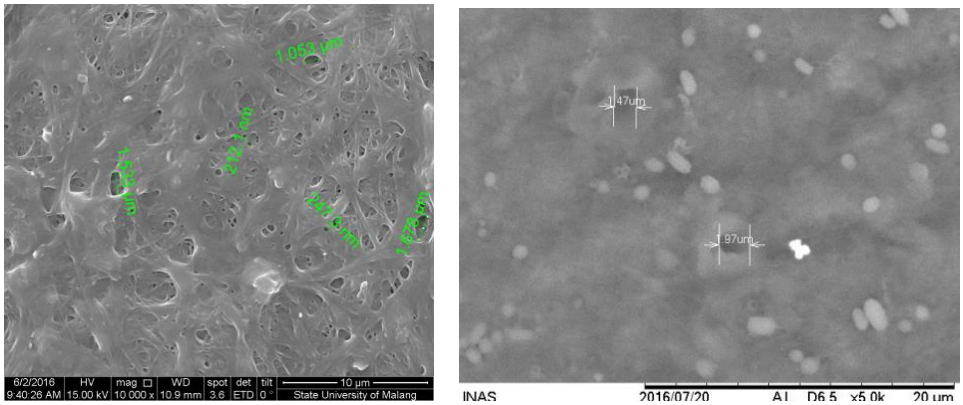

Figure 3. The morphology test result of the control sample (BC – Collagen) (b) The morphology test result of sample D (BC – Collagen – Glycerol 0.3%).

Tensile Strength Test

The tensile strength test was performed to determine the mechanical properties of the BC-Collagen-Glycerol sample by using Autograph Imada HV-500 NII. At first, the sample was formed in a dog-bone structure with a length of 63.5 mm, a width of 10 mm, and the length of the gauge of 5 mm based on the American Society for Testing Material (ASTM D 1822 L) for each variation. Then, the thickness of the sample was measured with a digital micrometer. Finally, the sample was mounted to the clamp and stretched with speed of 10mm/minute and maximum force of 50 N. The result of tensile strength shown in Figure 2.

The Morphological Test by using Scanning Electron Microscope (SEM)

The morphology test was performed to determine the pore size and membrane morphological structure of the tested material. Based on the result of the mechanical strength test, it was obtained that the best sample in term of elongation was sample D (Bacterial cellulose – collagen – 0.4% glycerol). Sample D with a magnification of 5000x has a pore size of 1.47 µm – 1.97 µm, while the control sample has a pore size of 1.53 µm -1.678 µm with the same magnification (Figure 3).

Cytotoxicity Test

Cytotoxicity test on control samples and BC-Collagen-Glycerol using MTT assay testing methods. In this test, using Baby Hamster Kidney cells (BHK)-21. Cytotoxicity test is a test to identify the toxicological properties of a material that is visible from his cell viability, which can be done using MTT assay testing methods. OD values were obtained from Elisa Reader is used to calculate the percentage of living cells from each sample. Figure 4 showed that the cell viability in a range of 78-93%.

The Degradation Test by using Simulated Body Fluid (SBF)

The degradation test was performed to determine the degradation ability of the artificial dura mater sample. This test was done as a simulation when the artificial dura mater is applied to the human body. The results shown in Table 2.

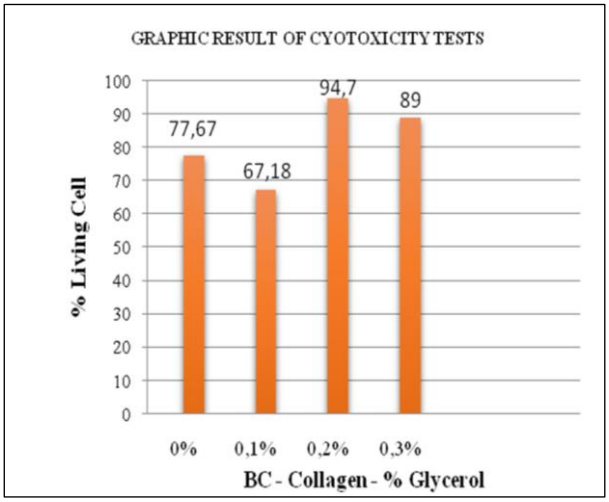

Figure 4. The Cytotoxicity test result of Bacterial Cellulose-Collagen-Glycerol.

Table 1. Tensile Test Results of PVC-Glycerol-Chitosan Biocomposite Membrane.

Sample	Plasticizer Concentration (%)	Fmax (N)	A ($mm^{2)}$	Elongation (%)	Tensile Strength (MPa)
A	0	3,375	0,11949	2,81	33,89
B	0,1	4,13	0,10587	2,3	23,4
C	0,2	3,235	0,09399	5,2	23,47
D	0,3	1,945	0,099	8,6	23,58

Table 2. The Result of Degradation Test

Variation	**Wo (gram)**	**Wt (gram)**		**% of Degradability**	
		7 Days	**14 Days**	**7 Days**	**14 Days**
A (BC–Collagen)	0.0033	0.0026	0.0021	21.21	36.36
B (BC– Collagen-Glycerol 0.1%)	0.0037	0.0036	0.0034	2.77	8.10
C (BC– Collagen-Glycerol 0.2%)	0.0033	0.0021	0.0013	36.36	60.60
D (BC– Collagen-Glycerol 0.3%)	0.0113	0.0096	0.0092	15.04	18.58

DISCUSSION

This study was focus on the concentration variation of glycerol, which was 0.1%, 0.2%, and 0.3%. This test was aimed to analyze the functional group and observe whether there is an interaction chemically or physically. The result of this test showed a sharp peak of spectrum that indicated the hydroxyl group in the range of 3600-3100 cm^{-1}. There was also a stretching amide group at 1075-1000 cm^{-1} that was an indication the presence of collagen. This result implied that the synthesis was successful (Figure 1).

Figure 2 is showed that the addition of glycerol could increase the elongation value the addition of glycerol as a plasticizer caused an increase in the elongation [11]. The elongation value of sample A (Control), B (0.1% glycerol), C (0.2% glycerol), and D (0.3% glycerol) were 2.81%, 2.3%, 2.8%, and 8.6%, respectively. The higher the concentration of the glycerol, the higher the elongation value because the glycerol would decrease the inter-molecules binding between neighboring polymer chains, thus the elongation increases. The bacterial cellulose-collagen without glycerol (the control sample) forms a linear polymer with covalent bonds while the one with glycerol produces a branched polymer with hydrogen bonds. The covalent bond is stronger than hydrogen bond. Thus, a weaker bond in the control sample leads to lower tensile strength too.[6]

The result of this study showed that the highest elongation value was 8.6% in sample D which was bacterial cellulose – collagen – glycerol 0.3%. Based on the study of Saska [12] about bacterial cellulose biocomposites, it was obtained that the elongation value or strain had a range of 7-14% for artificial dura mater. While Chauvet's study [13] showed that the strain of mechanical test on human dura mater has a maximum strain of 10%.

The ultimate tensile strength (UTS) was used to determine the material strength specifically which was the maximum tensile obtained before breaking or tearing (Table 1).

Table 1 is showed that the tensile strength was not decreased significantly. This might be caused by the low concentration of glycerol so that it did not show a significant effect on the tensile strength.

The control sample which was bacterial cellulose – Collagen had a UTS of 33.89 MPa. With the addition of 0.1%, the UTS reached a value of 23.4 MPa. Those value still did not meet the standard of artificial dura mater tensile strength which was between 0.6 MPa – 16 MPa [14]. This might be caused by the structure of collagen that could form a strong interaction in the bacterial cellulose [5]. The collagen has a complex structure and consists of fibrils that allow a formation of tissue that could interact with the other molecules. Collagen is one of protein that has a high tensile strength and also a material that has good elongation.[14] The tensile strength was affected by the measured surface of the sample. The homogeneity of sample surface also affects the tensile strength calculation. The difference in the sample surface could be caused by the difficulty to control the follicle thickness of the bacterial cellulose in the harvesting time due to the bacterial activities.

Next, samples characterized by morphologic structure using Scanning Electron Microscopy (SEM). The control sample showed that the pores were spread evenly in the surface of the membrane, while the treated sample showed a small number of pores. With a standard of artificial dura mater pore size of 1.5 μm-3.9 μm [15], it could be concluded that the bacterial cellulose – collagen – glycerol has a big potential as artificial dura mater because it met the standard of artificial dura mater pore size of 1-22 μm so that it might induce cell attachment and prevent the leakage of the fluid [14]. The addition of glycerol might be correlated with the interconnected pore size of bio-composite membrane [16]. The result of morphology test was shown in Figure 3.

Based on cytotoxicity test (seen in Figure 4), showed that the cell viability in a range of 78-93%. On the previous study, those value was non-toxic because it was above 50% [17]. That result showed that cell viability was above 50%, because the used material, bacterial cellulose was a microbial polysaccharide produced from fermentation process from Acetobacter xylinum that has the same chemical structure of plant cellulose. Collagen is also the main component of human body. The collagen is obtained from the fish skin which is a natural product so that it could be concluded that the dura mater is safe to use in the human body.

Degradation test used SBF solution that has been made based on the study of Kokubo et al [7]. The SBF solution had a pH of 7.33 and then the sample was immersed from 7 and 14 days to observe its behavior in that environment. The mass loss was measured as a degraded mass. The result was shown in Table 2.

The degradation process was taken place slowly, piece by piece. The degradation test on the control sample (Bacterial Cellulose-Collagen) showed a result of 21.21% on the 7th day and 36.36% on the 14th day. The degradation test on Sample A (Bacterial Cellulose-Collagen-0.1% Glycerol) showed a result of 2.77% on the 7th day and 8.1% on the 14th day. The degradation test on Sample B (Bacterial Cellulose-Collagen-0.2% Glycerol) showed a result of 36.36% on the 7th day and 60.65% on the 14th day. The degradation test on Sample C (Bacterial Cellulose-Collagen-0.3% Glycerol) showed a result of 15.04% on the 7th day and 18.58% on the 14th day.

The result is indicated that Sample C (Bacterial Cellulose-Collagen-0.3% Glycerol) was the optimum one because it met the standard of artificial dura mater for head trauma case. Not only from degradation test result, but also the other characterization result showed that it had O-H stretching functional group which showed the presence of glycerol in the cellulose sample I and C-O stretching bond of 1053.85 cm^{-1} that indicated the amide I group from collagen. The tensile strength with elongation value that met the standard of artificial dura mater. The morphology test result showed the pore size that met the standard of

artificial dura mater pore size and the percentage of living cells in the toxicity test could reach 94.7%.

CONCLUSION

The FTIR test result is showed that the most dominant interaction was between the hydroxyl group in the bacterial cellulose I and glycerol. The tensile strength test is showed that bacterial cellulose – collagen – 0.3% glycerol is the sample in accordance with the standard of elongation of artificial dura mater which was 7-14%. The morphology test showed that sample D (BC – Collagen – Glycerol 0.3%) is also suited the pore size standard of artificial dura mater. The cytotoxicity test result indicated that the samples were non-toxic.The most optimal sample to be used as an artificial dura mater was sample with bacterial cellulose – collagen – 0.3% glycerol.

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