Citric Acid-Polyurethane-Chitosan-Based Innovative Biocomposites as Candidates of Antibacterial Dialyzer Membrane

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Abstract

Hemodialysis treatment is a replacement therapy renal function. Hemodialysis therapy with reuse system causes a risk of bacterial infection. In addition, routine vascular access by hemodialysis patients also factors of bacterial contamination which can increase the comorbidity and mortality rates of patients. This research aims to obtain biocompatibility and antibacterial properties by adding concentration chitosan in three variations. The functional group test showed the presence of a bond between PU-CA-PES-CS at a certain wavelength. SEM test proved that the pores produced on dialyzer membrane are still within the size range for the hemodialysis application at 0.001-0.1 µm. Hemolysis test results showed that the percentage of hemolysis in Sample A, Sample B, Sample C, and Sample D were 4.18%, 3.34%, 2.30% and 1.67% respectively. The percentage of hemolysis at less 5% is safe for contact with blood. Antibacterial test results revealed that PES-PU-CA membrane samples coated with chitosan variation are antibacterial. It can be seen from the formation of clear zone around the sample disc with diameter of 6,946-11,086 mm. PES-PU-CA dialyzer membrane coated with chitosan is a safe composite and potentially applicable as an antibacterial dialyzer membrane based on functional group test, antibacterial test, hemolysis assay, SEM, and cytotoxicity test.

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Introduction

Chronic Kidney Disease (CKD) or chronic renal failure is a condition in which kidneys have a lymphatic lesion or glomerular filtration rate (<60 mL/min) within 3 months or more.[1] Kidney damage is evident from abnormalities in blood, urine, or renal biopsy. Chronic renal failure is one of the major problems in medical world in which the incidence rates continue to increase and require expensive maintenance costs. In 2000, CKD ranks eight out of ten for the most common causes of death in the world.

Hemodialysis treatment is a therapy to replace kidney function and should be performed continuously; thus, it costs a lot.[2] One way to reduce its cost is by reusing dialyzer which can be used up to 7 times for the same patient. However, there is a disadvantage of reusing, namely bacterial contamination, possible transmission of infectious agents, and chemical-related complaints which are used in repeated dialyzer.[3,4]

Biopolymers, which are potential to be developed as a basic material of hemodialysis membranes, are polyurethane, because it is highly resistance to protein absorption and able to increase membrane compatibility.[5] Anti-coagulant dialyzer membranes without heparin were investigated by performing citric acid transplantation reactions on polyurethane.[5] Citric acid (CA) is one of the more commonly used and more affordable anticoagulants than heparin. Modified polyethersulfone synthesis resembling heparin surfaces and exhibited improved hydrophilicity, good compatibility, potential to use in blood purification (hemodialysis), and stability at high temperatures.[6]

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Another important thing to consider after the hemodialysis process is the infection occurred in patients. Infections in hemodialysis patients may come from the water source, water treatment system at the dialysis center, water distribution system, dialysate fluid, dialysis machines, and regular vascular access.[7] From these cases, antibacterial dialyzer membrane is needed for hemodialysis patients. Adding Chitosan in formulating dialyzer membrane can result in antibacterial character.[6,7] Chitosan is one of the most abundant polymeric materials in nature, good antibacterial, and hemostatic. In addition, Chitosan also has natural water absorption ability, because its main chain contains amino, hydroxyl, and chemically active.[9] Chitosan molecule that could improve its antimicrobial properties, and the physical state of the chitosan, e.g. whether it is present in the form ofhydrogels, powders, films, membranesmicro/nanoparticles and coatings.[10] The resulting membrane from Chitosan mixture has a strong biocidal effect on bacteria. Characterization used included FTIR test, cytotoxicity test, SEM test, hemolysis assay test, and antibacterial test.

Materials and methods

Tools and materials

The tools used include digital balance, glass beaker, magnetic bar, magnetic stirrer, spinneret, spatulas, and aluminum foil. Characterization tools include MTT Assay (Elisa reader), SEM (Phenom), waterbath, sentrifuge, spektrofotometri UV vis. The materials used in this study are polyurethane, chitosan, polyethersulfone, citric acid, dimethylformamide, and distilled water.

Synthesis of Membrane Dialyzer

Preparing polyurethane (PU), citric acid (CA), polyethersulfone (PES), chitosan (CS), and dimethylformamide (DMF). Two percent of polyurethane (PU) and 2% of citric acid (CA) were diluted in 78% dimethylformamide (DMF) at 80°C for 4 hours to form PU-CA. Then, in PU-CA solution, 18% of polyurethane (PES) was added so as to form dope solution ready to be printed in 2 forms, flat and hollow fiber. In this research, three (3) variables were used: control variables (CA 2% -PU 2% -PES 16 wt%), independent variables (3 variations of CS concentration of 0.25% (w/v); 0.35% (w/v); and 0.50% (w/v), and dependent variables (CA-PU-PES composite characteristics as dialyzer membrane).

Membrane's printing process with a flat shape was conducted by pouring dope solution in a petri dish, then dipped into the water; while for hollow fiber, it was conducted using spinneret tool as presented in Figure 1.

The printed membranes were then dipped into Chitosan solution with concentration variations of 0.25; 0.35; 0.50 for 24 hours at 4°C. Then, the membrane was washed using Phosphate Buffer Saline (PBS) and was purged for 30 minutes in glutaraldehyde. Characterization of functional group test, antibacterial test, SEM test, hemolysis test, and cytotoxicity test were conducted afterwards.

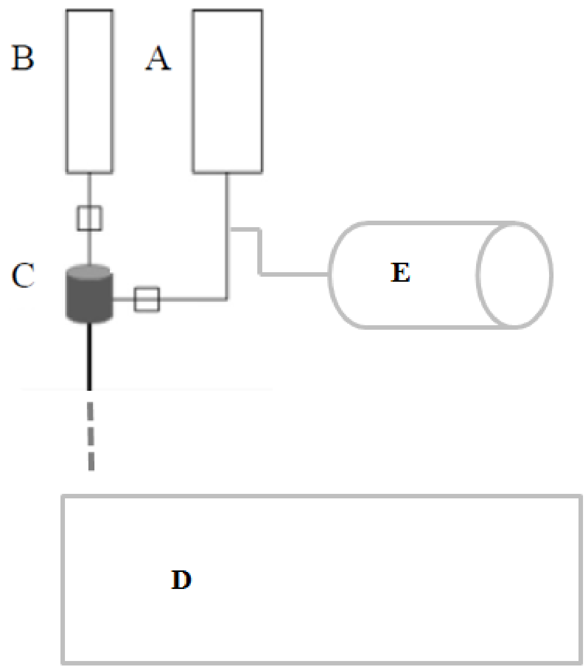

Figure 1. Scheme of Hollow Fiber Membrane Print Process with spinneret tool. A. Dope Tubes, B. Water Tubes, C. Spinneret, D. Coagulation Tube, E. Gas Tube

Result and Discussion

This research has successfully synthesized membrane dialyzer with polyurethane base material to react with citric acid. Then polyethersulfon was also added in it, so it can produce good film structure. Finally, the membrane was coated with Chitosan solution to elicit antibacterial character.

Functional Group Test

Functional group test aims to find out the functional groups contained in the synthesized blood sample. Based on test results using Fourier Transform Infrared (FTIR) in Table 1, there are PU-CA-PES-CS composite bonds of the OH- and -NCO functional groups in PU with wavelength of 2300 and 2250 cm^{-1}; CA numbers of OH- with wavelength of 2800 and 2929,87 cm^{-1}; PES of SO_2 functional group with wavelength of 1151,50 and 1153,43 cm^{-1}; and CS from NH and OH functional group with wavelength length of 3066,82cm^{-1}.

Sample	OH- and –NCO Group	OH- Group	SO_2 Group	N-H/ O-H Group
Without Chitosan	2300	2800	1151,50	-
With Chitosan	2250	2929,87	1153,43	3066,82

Table 1. Functional Group Test Results.

Antibacterial Test

Antibacterial test aims to determine the response of membrane dialyzer to bacterial activity. The tests were performed using Staphylococcus aureus bacteria and repeated 3 times. Agar used for bacterial culture nutrition was Trypticase Soy Agar (TSA). The antibacterial activity of Chitosan is derived from amine functional group which can form bonds with bacterial and bacterial cell walls to lysis [8]. Based on the test results, it was revealed that PES-PU-CA membrane samples coated with Chitosan are antibacterial. It can be seen from the formation of clear zone around the sample disc with a diameter of 6.946-11.086 mm (as shown in Figure 2). The optimal sample is recorded by the highest Chitosan-concentrated sample at 0.50 mg. The interaction between Chitosan and outermost cell membrane of bacteria affirms that concentration influences antibacterial character. The higher concentration is, the greater the inhibition of bacteria will be.[11]

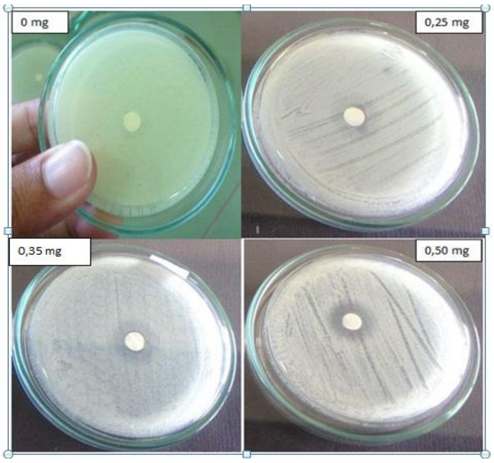

Figure 2. (a)

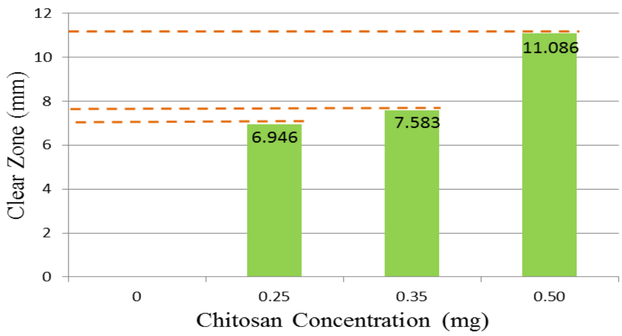

Figure 2. (b)

Figure 2. (a) Antibacterial test (b) Bar chart of the test result.

Hemolysis Test

Hemolysis results are based on erythrocyte lysis caused by contact, toxic, metal ions, or other causes which can damage erythrocyte. The test was performed using human blood samples, sterile aquades, and saline. Blood taken and dissolved in aquods and saline. Then it was incubated in water bath at 37°C for 2 hours. Next, blood in microtube is centrifuged at 3000 rpm for 10 minutes. The supernatant formed was taken and transferred to a micro well plate to test its absorbance using UV-Vis spectrophotometry with 490nm wavelength. The test was conducted in 3 times iteration. Based on the test results, the percentage of hemolysis in Sample A, Sample B, Sample C, and Sample D were 3.34%; 3.18%; 2.30%; and 1.67% respectively. The percentage of hemolysis is less than 5% and deemed safe for contact with blood,[12] so all samples are safe for contact with blood. Increased Chitosan concentration also affects the decreasing percentage of hemolysis (as shown in Figure 3).

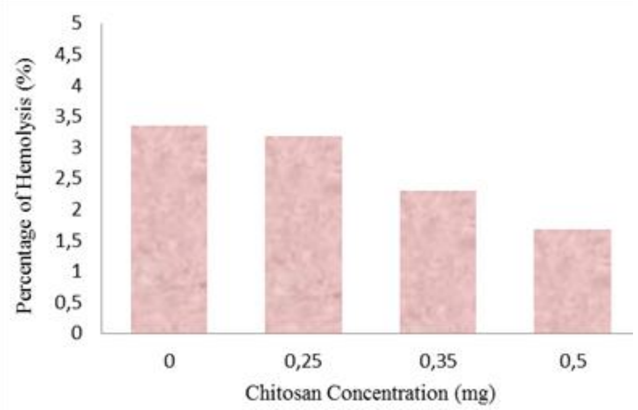

Figure 3. Increased chitosan concentration causes the percentage of hemolysis to decrease.

Morphology Test

SEM test aims to determine membrane morphology, which can be known from its structure and pore size. Therefore, based on the data of SEM test results, uniformity of usable membrane structure can be determined. SEM test was conducted using Perox. The samples were placed on a holder and inserted into the recorder tool to be displayed on computer screen (as shown in Figure 4). Sample used for this test is the best sample with the highest concentration of Chitosan (0.50 mg). SEM test for topography illustrated that the pore size of dialyzer membrane surface was 0.095 µm, as shown in Figure 4. The pore size that can be used in the application of hemodialysis is the ultrafiltration pore size at 0.001-0.1 µm.[13] Hence, the pore produced were still within the range for hemodialysis application.

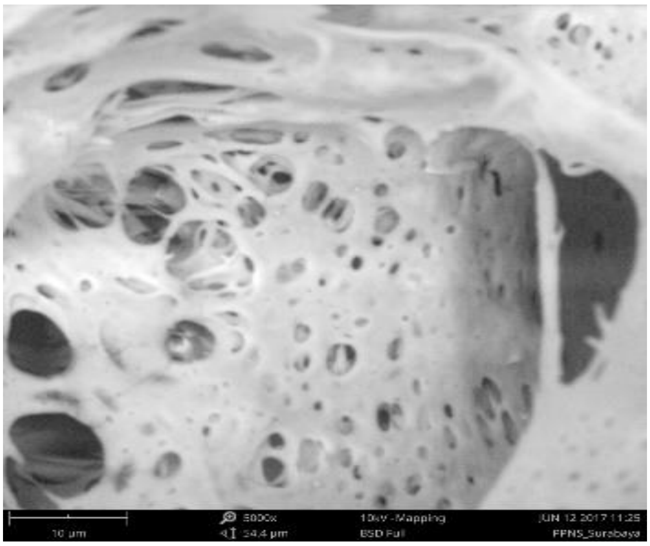

Figure 4. SEM Test Results.

Cytotoxicity Test

Material toxicity level was tested using MTT assay method. Samples were incubated on erythosit cells in eagle's medium for 24 hours. To determine the percentage of his living cell by determining the absorption wavelength using ELISA Reader. The result could be seen at Figure 5.Material is not toxic if the percentage of living cells is more than 50%.[14] Based on the cytotoxicity test through MTT Assay method showed that the four samples are not toxic.

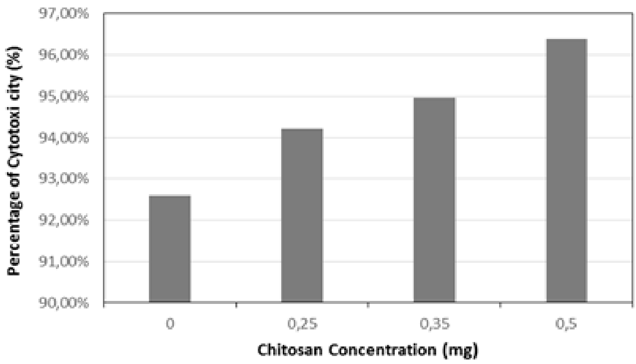

Figure 5. Increased chitosan concentration causes the percentage of cytotoxicity to increase.

Conclusions

Biocomposites of citric acid (CA)-polyurethane (PU)-polyurethane (PES)-Chitosan (CS) can be used as antibacterial hemodialysis membrane candidates based on test results which have been performed, namely functional group test, antibacterial test, haemolysis test, SEM test, and cytotoxicity test.

Acknowledgements

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