Making Hydrogel with Crosslinked Reactions between Chitosan and Dialdehyide Cellulose from Coconut Fiber as Wound Healers

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Abstract: Making hydrogels from crosslinked reactions between cellulose oxidized with chitosan through Schiff base formation reaction has been investigated as a wound healing drug in vivo. α-Cellulose obtained from the isolation of coconut fiber by 14.24 g. α-Cellulose is oxidized by using KIO_4 to be dialdehyde cellulose. The degree of oxidation of cellulose dialdehyde is 86%. Hydrogels were made by schiff base crosslinking reaction between chitosan and dialdehyde cellulose with temperature variations of 75, 100, 125 and 150 oC. The optimum temperature in hydrogel synthesis is 100 oC. the formation of a hydrogel is supported by the presence of FTIR Spectrophotometer where the spectrum of 1643.35 cm-1 is formed where the group -C=N- which shows the formation of a Schiff base reaction. The hydrogel that is obtained has a good swelling ability of more than 1000%. Invivo analysis was carried out for 7 days in mice and as a result, the injured mice have recovered and have not left a mark.

1 INTRODUCTION

Coconut coir is one of the biomass that is easily obtained and is a by-product of agriculture. The coir composition in coconuts is about 35% of the overall weight of the coconut fruit. Coconut coir consists of fiber (fiber) and cork (pitch) which connects one fiber to another fiber. Coconut coir consists of 75% fiber and 25% cork. Coconut coir fibers contain lignin (35% - 45%) and cellulose (23% -43%) (Carrijo, et al., 2002).

Cellulose is a linear condensed polymer composed of D-anhydroglucopiranose units bound by β-1,4-glycosidic bonds (Kalia, 2011). Cellulose has one reducing group containing a non-substituted hemiacetal, and one non-reducing group containing an additional hydroxy group in C4. There are 2 main crystalline cellulose arrangements, namely cellulose I and cellulose II. Almost all initial cellulose consists of cellulose I. Cellulose which has been dissolved and deposited (regenerated) or through treatment with concentrated alkaline solution and rinsed with water (mercerized) consists of cellulose II. The change in cellulose I to cellulose II is irreversible (Wertz, 2010).

Chitosan is a natural polysaccharide obtained from chitin deacetylation. If most of the acetyl groups in chitin are substituted by hydrogen atoms into amine groups by the addition of a strong high concentration base solution, the result is called deacetylated chitosan or chitin (Bastman, 1989). Chitosan is one of the sources of amino natural polysaccharides known as pH-sensitive properties (Zhao, 2003).

Cellulose dialdehyde can be produced by reacting cellulose with potassium periodate. Calcium periodate is a selective oxidizer that will only break the C2-C3 bonds so that 2 aldehyde groups are formed (Höglund, 2015).

Hydrogels are essentially cross-linked polymers which have the ability to absorb water thousands of times from their dry weight, but are not soluble in water due to the presence of a 3-dimensional structure on the polymer network. Hydrogel is a very interesting material because of its unique solubility and water carrying capacity (Erizal, 2010).

The In vivo process is divided into three phases, namely:

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The inflammatory phase lasts from the time of the wound until the third day. Broken blood vessels in the wound cause bleeding and the body will try to stop it with vasoconstriction. Hemostasis occurs because platelets that come out of blood vessels stick together and together with fibrin formed freezes blood coming out of blood vessels.

The proliferation phase is also called fibroplation because what stands out is the process of fibroblast proliferation. In this phase the fiber is formed and destroyed again to adjust to the stress on the wound that tends to constrict. This property, together with the contractile properties of miofibroblasts, causes attraction to the edges of the wound. At the end of this phase the wound strain strength reaches 25% of normal tissue. Later, in the process of increasing the strength of collagen fibers increases due to intramolecular bonds and between molecules. In this fibroplasias phase, the wound is filled with fibroblasts and collagen which forms a reddish colored tissue with a fine-grained surface called granulation tissue. The wound edge epithelium consisting of basal cells is released from the base and moves to fill the wound surface. The place is then filled with new cells formed from the mitotic process. The migration process can only occur in a lower or flat direction, because the epithelium cannot migrate in a higher direction. This process only stops after the epithelium touches and closes the entire wound surface. With the closed surface of the wound, the process of fibroplasia with the formation of granulation tissue will also stop and the maturation process begins in the phase of completion.

In this phase there is a maturation process that consists of excessive tissue reabsorption, shrinkage and finally new tissue is formed. The body tries to normalize everything that is abnormal because of the healing process. During this process, the scar tissue is pale, thin and weak and easily moved from the bottom. Maximum shrinkage is seen in wounds. At the end of this phase, skin wound healing can withstand stretches of approximately 80% of normal skin ability (Moenadjat, 2003).

2 MATERIALS AND METHODS

2.1 Tools

The tools used in this study include: glassware, beaker glass, sieve, petri dish, reaction tube, analytical balance, oven, hot plate stirrer, thermometer, desiccator, incubator, Fisher Scientific, vacuum device, universal indicator, Aluminum foil, Thermometer , pH meter, Alcohol Meter, FTIR, SEM.

2.2 Materials

The materials that used in this study include: Coconut coir, Chitosan, Water, Aquadest, HNO_3, $NaNO_2$, NaOH, Na_2SO_3, NaOCl, H_2O_2, KIO_4, Acetate buffer, $KMnO_4$.

Chitosan powder (1, 2, 3 and 4 g) was dissolved in a 100 ml acetic acid 0.2 M solution at 60 ºC and 600 rpm to produce chitosan solution. Agar powder (5 g) was dissolved in a 100 ml deionized water at 95 ºC and 600 rpm for 60 minutes. An amount of 20% of glycerol was added to the agar and chitosan solution as plasticizer. The agar-chitosan film was fabricated by mixing chitosan solution (1, 2, 3 and 4 g/100 mL) and agar solution (5 g/100 ml) with a ratio of 1:1. The mixture was stirred at 60 ºC and 600 rpm for 60 minutes. An amount of film solution was distributed into the template for drying and casting for 24 hours at 40 ºC. The films were stored in a desiccator.

2.3 Research Procedure

2.3.1 Preparation of Coconut Fiber Powder

Coconut coir is separated from the outer skin. Then the fiber is washed with running water. Then the clean coconut fiber is dried in the sun for 1 day. Dry coconut coir is cut into smaller pieces and then in a blender. Then the coconut husk that has been finely filtered uses an 80 mesh sieve.

2.3.2 Isolation of α-Cellulose from Coconut Coir

Weighed as much as 75 grams of coconut fiber powder is put into a glass beaker, then added 2000 ml of 3,5% HNO_3 and 10 mg $NaNO_2$ then heated at 90 ℃ for 2 hours while stirring on a hot plate. Filtered and washed residue until neutral filtrate. Then added 375 ml of 2% NaOH and 375 ml of 2% Na2SO3, heated at 50 ℃ for 1 hour while stirring on the hot plate then filtered and washed away until neutral filtrate. Then it was bleached with 500 ml of 1.75% NaOCl solution, heated at 70℃ for 30 minutes while stirring on a hot plate. Filtered and washed residue until neutral filtrate. Then added with 500 ml of 17.5% NaOH and then heated at 80℃ for 30 minutes while stirring on a hot plate. Filtered and washed residue until neutral filtrate.

Then added with 250 ml of 10% H_2O_2, heated at 60℃ for 15 minutes while stirring on a hot plate. Filtered and washed residue with aquadest until the filtrate is neutral. Dried the residue in the oven at 60℃ then stored in the desiccator. (Ohwoavworhua, 2009)

2.3.3 α-Cellulose Oxidation with Potassium Periodate (KIO_4)

1 gr of isolated cellulose was immersed in a mixture of KIO_4 solution and 0.1 M acetate buffer, with a ratio of KIO_4 and acetate buffer 1: 100 (b / v) and variation of KIO4 concentration of 0.2; 0.4; 0.6; 0.8; 1.0 mg / ml. Then stir it slowly without light and the reaction conditions pH 4.5 and temperature 40o C for 60 minutes. After oxidation, it is washed with aquadest. The oxidized cellulose is characterized by FT-IR. (Liu, 2004)

2.3.4 Determination of the Degree of Oxidation (D.O) of Cellulose

A total of 0.1 grams of sample was dissolved with 10 ml of aquadest. Then added with 10 mL of NaOH 0.1 N. The solution is then heated to complete dissolution. After that, the solution is cooled. Added to the 10 mL 0.15 N HCl solution to the pH of the solution <7. Then add aquadest as much as the volume decreases during the first heating. The solution is reheated for 1 minute, then the PP indicator 2 drops is added. Titrated with 0.1 N NaOH solution, and observed color changes. Determination of the degree of oxidation can be determined through the following equation:

$$\%DO=((C_{NaOH} \times V_{NaOH})-(C_{HCl} \times V_{HCl}))/(m/162)\times 100\%$$

2.3.5 Crosslinking between Oxidized Chitosan and Cellulose

Chitosan solution was made by: as much as 8.0 grams of chitosan were put into 400 ml of acetic acid solution 2% (v / v) with constant stirring for 1 hour at 60oC then oxidized cellulose was immersed in chitosan solution with stirring for 15 minutes at sushu 60 ºC so that a thick brownish yellow solution is formed. Then poured into a glass beaker and dried at a temperature variation of 75, 100, 125 and 150 ºC for 2 hours. (Pratama, A 2018)

2.3.6 Characterization of Hydorgels Chitosan-dialdehyde Cellulose

FT-IR Analysis.

The sample is prepared in the form of pulp. Porridge is examined in a thin film placed between flat plates of salt. The test is done by clamping the mixed film on the sample site. Then the film is placed on the plate in the direction of infrared light. The results will be recorded periodic paper in the form of a wave flow curve 4000-200 cm-1 to the intensity.

Test of Water Absorption Percentage.

Testing the percentage of water absorption was carried out by determining the percent swelling ratio by measuring the initial weight (Wd) of the sample which was then immersed in distilled water for 24 hours. The soaked samples are then filtered using filter paper and measured the final weight (Ws). Measuring the percentage of water absorption in the hydrogel can be determined by the following formula (Muthoharoh, 2012):

$$\%\ S=(Ws-Wd)/Wd\ x100\ \% \quad (1)$$

Where :
% S = Percentage of water absorption (%) (g/g)
Ws = Swollen hydrogel weight (g)
Wd = Dry weight of hydrogel (g)

Cross-linking Test.

The percentage value of crosslinking can be done by determining the crosslink percent percent where the dry weight of the resulting hydrogel is weighed. Then the hydrogel is soaked with a solvent (chloroform) for 24 hours. After immersion, the hydrogel is heated at a temperature of 60oC to dry for 3 hours. The dry weight of the hydrogel after immersion is determined by weighing using an analytical balance. The degree of crosslinking can be determined by the following formula (Muthoharoh, 2012):

$$\%\ DC= Wg/(Wo\ ) \times 100 \quad (2)$$

Where,
% DC = degree of crosslinking
Wg = Dry weight of hydrogel (g)
Wo = Swollen hydrogel weight (g)

Morphological Analysis using Scanning Electron Microscope (SEM).

The microscopic observation process using SEM begins with glueing the sample with a stick made of older metal specimens. Then the sample is cleaned with a blower, then the sample is coated with gold and palladium with a dionspater machine pressurized $1492x10^{-2}$ atm. The sample is then put into a special room and then illuminated with 10 Kvolt-powered electrons so that the sample emits secondary electrons and the electrons are detected by a scientor detector which is then amplified by an electric circuit that causes a chatode ray tube (CRT) image. Shooting is done after selecting a specific part of the object (sample) and the desired magnification so that a clear photo is obtained (Negulescu, 2004).

Antimicrobial Activity.

Antimicrobial activity of hydrogels was performed on two types of microbes, i.e., S. Aureus and E. Coli. The microbes culture were diluted in accordance with the standards McFarland, each inoculated into petri dishes containing Mueller Hinton agar. Then the disc was inserted into the blends of dialdehyde cellulose/chitosan in a petri dish aseptically. The petri dish then put in an incubator at 35 °C for 24 h. After 24 h, the antimicrobial zone (clear zone) was observed and measured in diameter by using a caliper.

In Vivo.

Before cutting, the hair around the femur was shaved and anesthetized using lidocaine 0.2 cc in 2 cc aquadest, then injured. The injury is done at the femur of the mouse by making an incision with a length of 20.0 mm and a width of 1.8 mm using a sterile scalpel then handled. Handling is done twice a day, before giving the sample to the wound, always clean it first using distilled water. Giving the sample is done by applying it to the part of the wound on the femur of the mice, ie in the morning and evening, for 7 days after induction of the wound using a cotton bud. As a comparison, negative controls and positive controls were used using Salticin Gentamicin Sulfate.

3 RESULTS AND DISCUSSIONS

In this study α-Cellulose can be isolated from coconut fiber waste. Where from 75 grams of coconut coir powder isolated 14.24 grams of pure cellulose were obtained or about 18.98% of the initial mass of coconut husk samples used
α-Cellulose is oxidized using potassium periodate which is a selective oxidizer. Potassium periodate will break the C2-C3 bond which has a secondary alcohol group so that it becomes 2 aldehyde groups.

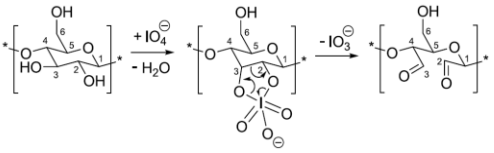


Figure 1: Cellulose Oxidation Reaction with Periodate.

The reaction between watershed and chitosan is an imine group formation reaction (Schiff base), where the primary amine group in chitosan will react with carbonyl aldehyde groups found in the watershed to form an imine group (R-C = N-).

3.1 FT-IR Analysis

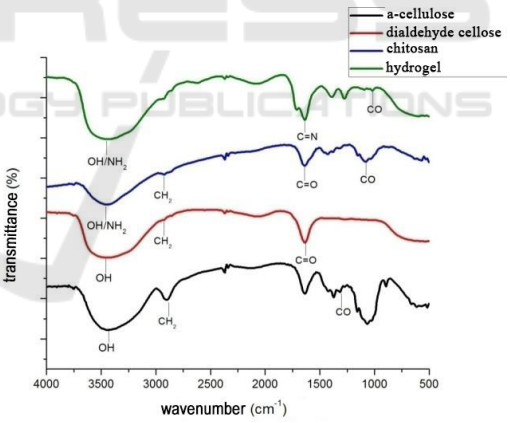


Figure 2: Spectrum FT-IR α-Cellulose, DAS, Chitosan, Hydrogel.

The results of the analysis of the α-cellulose functional group used for the study showed that the absorption of wavelengths of 3448.72 cm^{-1} showed the presence of a bound -OH group found in α-cellulose compounds, the absorption of a wavelength of 2900.94cm^{-1} indicated the presence of a group -CH sp3 and the absorption of wavelengths of 1064.71cm^{-1} indicates the presence of -CO- groups.

The results of the analysis of the cellulose dialdehyde (DAS) functional group used for the study showed that the wavelength absorption of 3441.01cm^{-1} showed the presence of a bound -OH group found in cellulose dialdehyde compound (DAS), with wavelength uptake of 2924.09cm^{-1} shows the presence of -CH sp3 group and the presence of wavelength uptake of 1026.15cm^{-1} shows the presence of -CO- group and at wavelength absorption of 1635.64cm^{-1} indicates the presence of –C = O-.

In the FTIR spectrum of chitosan powder, there was an absorption peak at wave number 3448.72 cm^{-1} which showed the free peak absorption of O-H and nitrogen amine (-NH_2). With the presence of N-H absorption peaks, it is the main characteristic of chitosan structure. In addition, the presence of absorption peaks in wave numbers can also be referred to as marking N-H stretching primary aliphatic amines (Rohman A, 2014). According to (Fessenden and Fesssenden, 1982) if there are two hydrogens on a nitrogen amine (-NH_2), absorption of N-H appears as a twin peak. This is not proven by the results of the FTIR spectrum of chitosan powder analyzed showing that there were no twin peaks on the absorption band around 3000 - 3700 cm^{-1}. In addition there is an absorption peak at wave number 2924.09 cm^{-1} indicating the presence of –C-H sp3 bond. Furthermore, the absorption peak at wave number 1265.30 cm^{-1} indicates the presence of C-O and C-N groups. The absorption bands of C-O and C-N groups are usually not easy to identify in the fingerprint area because this spectrum area often contains many peaks that overlap and are difficult to identify (Fessenden and Fesssenden, 1982). But on the results of the spectrum analysis of chitosan powder used for research, CO and CN fingerprint areas are easy to identify. One of the factors that supports this convenience is that chitosan powder used in the study can be categorized as pure. In addition, where this CN group is also a characteristic typical functional groups found in chitosan molecules. From the description above, it can be seen that the results of functional group analysis using FTIR spectrophotometer on chitosan powder have no changes in the functional groups composed of chitosan chemical molecules or there are no other groups that absorb infrared absorption from FTIR spectrophotometers so that chitosan powder used in research is pure chitosan compound.

On the results of the hydrogel functional group analysis used for the study, the wavelength uptake of 3441.01 cm^{-1} showed the presence of a bound -OH group found in the hydrogel compound, the wavelength absorption of 2924.09 cm^{-1} indicated the presence of the -CH sp3 group . In the crosslinking reaction between α-cellulose with chitosan, an imine bridge (Bases Shiff) is formed as a change that links between chitosan compounds and α-cellulose. The C = N group causes vibrations on wavelength absorption of 1643.35 cm^{-1} but unlike chitosan uptake in the previous FTIR results, the absorption of these wavelengths does not form a twin peak because there is no hydrogen group in the nitrogen atomic bond chain, replaced by a bond duplicate between the carbon chain on cellulose dialdehyde and nitrogen in chitosan compounds. The occurrence of crosslinking process is also reinforced by the formation of uptake at a wavelength of 1273.02 as an amplifier of carbon atoms in the chitosan group still bound to nitrogen as from chitosan compounds. Similar to the results of the previous functional analysis, there was an absorption peak of 3441.01 nm as the absorption peak of the OH group, 2924.09 nm and 2854.65 nm as the absorption peak of the C-H sp3 and 1064.71 groups as the peak C-O-H absorption.

3.2 Percentage of Water Absorption

In making the hydrogel, the crosslinking reaction works well. With the ability to expand well, the air will be absorbed in crosslinked molecules between cellulose dialdehyde and chitosan. On heating 75^{o}C produces a percentage of water absorption below from heating 100 ^{o}C which is 3.91% g / g. This is evidenced by the destruction of the hydrogel into a cluster of fine grains proving that the crosslinking reaction between cellulose and chitosan dialdehyde has not been fully formed because the temperature of the crosslinking has not been fully formed. At an increase of 125 ^{o}C, and 150 ^{o}C, the percentage of air absorbency was lower than that of making hydrogels with an increase of 100 ^{o}C while the percentage of overall air absorption reached 800.73% g / g and 198.26% g / g. This shows a high increase. A perfect crosslinking occurs at 100 ^{o}C. It has started to repair or cut off the crossing. Because with cross bonding between polymers, it will allow bond bonding with the polymer where the air is absorbed in the hydrogel crosslinked polymer polymer. With the ability to absorb air, the results of this research hydrogel can be used as a media to store drugs, namely hydrogels as a healing agent for wound healing and even wound healing agents.

3.3 Percentage Crosslink Degree

On the results of the crosslinked degree test, the highest percentage of crosslinking was at 150 ºC heating. This is very inversely proportional to the percentage of water absorption. Where a good hydrogel is a hydrogel with the ability to absorb water high due to the optimum crosslinking and does not make the shape of the hydrogel hard and stiff so that good cross-linking capability will increase the ability of high water absorption.

At 75 ºC heating, the ability of crosslinking is the lowest because an imperfect crosslinking process occurs reinforced in the explanation of the ability of the absorption of water on the heating. With the modification of the polymer chain extension, chloroform as an extracting agent for polymeric materials is not able to extract hydrogel material with good cross-linking ability. Whereas at 75 ºC heating, the ability of low water absorption makes the average percentage of crosslinking at 18.94% g / g. The ability of chloroform to extract the remaining ingredients of the formula for making hydrogels, resulted in the shape of the hydrogel after extracting it with chloroform not decreasing its shape and weight after extracting it from its initial weight or dry weight. This is indicated by the results of crosslinking in the middle of the average percentage of cross-linking at 100 ºC heating which is 78.32% g/g. This shows that every gram of hydrogel formed, the crosslinking ability is in the range of 78.32% which is successfully cross-linked in the formula for making it.

Whereas the heating of 125 ºC and 150 ºC has a high percentage of crosslinking capacity, namely 91.61% g / g and 92.93% g / g. The high percentage of crosslinking does not indicate that the hydrogel is in a good shape. In fact, the hydrogel is rubbed on each side so it is possible that the hydrogel has been crystallized so that it is difficult to extract by chloroform.

3.4 Sem Analysis

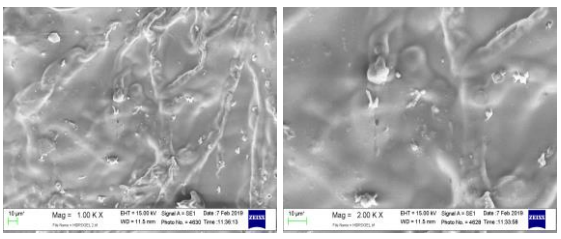
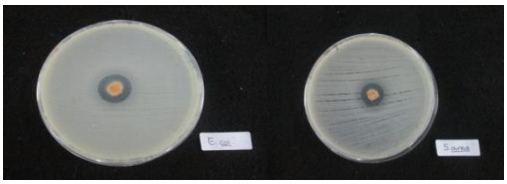


Figure 3: Results of morphological analysis of hydrogels using SEM with magnifications of 1000 and 2000 time.

3.5 Anti Bacterial Test Results with Hydrogels

Table 1: Antibacterial Test Results for Hydrogels.

	Diameter of hydrogel (mm)	Diameter of inhibition zone (mm)
E. coli	6.5	17.45
S. aureus	6.5	15.20



(a) **(b)**

Figure 4: observations of clear zones (a) Escherichia coli, (b) Staphylococcus aureus.

From Figure 4 it can be seen that hydrogels made from crosslinked reactions between chitosan and watershed have good antimicrobial activity against Escherichia coli bacteria as gram negative bacteria and Staphylococcus aureus bacteria as gram-positive bacteria.

3.6 *In Vivo*

Inflammatory phase is the phase where bleeding occurs and freezing or cessation of bleeding due to contraction of smooth muscle walls of blood vessels that are open and blood clots by thrombin and fibrin. The results showed that the inflammatory phase for control (-), hydrogel samples and control (+) had different healing phases. For control (-) takes more than 7 days, the control treatment (+) takes 7 days to heal but leaves a mark and not all mice tested recovered, while the treatment for hydrogel samples only takes 7 days to heal and does not leave made an impression.

(a) **(b)**

Figure 5: (a) Heal (without trace), (b) Heal (with trace).

4 CONCLUSIONS

From the results of the research that has been done, it can be concluded as follows:

The synthesis of hydrogels from crosslinking reactions between α-cellulose and chitosan tellah was successfully carried out. The crosslinking reaction forms an imine bridge (Schiff base) as a change that links between chitosan compounds and α-cellulose. This can be proven from the C = N group formed which causes vibration on wavelength absorption of 1643.35 and also has good water absorption capability.

The results of the antibacterial activity of the hydrogel showed good results for Gram-positive bacteria Staphylococcus aureus and gram-negative *E. coli* bacteria. This can be proven from the formation of clear zones around the sample.

Hydrogels show better In vivo efficiency than the healing drug Salticin Gentamicin Sulfate which is within 7 days and leaves no trace.

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