PLGA-Collagen with *Citrus paradise* extract as Antibacterial Absorbable Surgical Suture

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Abstract

Indonesian Central Bureau of Statistics in 2013 is reported that there were 100.106 cases of people getting injured because of accidents. The healing process of the injuries require surgical sutures to unify the wound. Suture either absorbable or non absorbable has possibility to be contaminated by pathogenic microorganism.Thereby, this condition could inhibit tissue healing which might lead to morbidity.This study aim is to make suture which absorb easily and has the antibacterial potency. The suture was made from PLGA – collagen and Citrus paradisi extract. The multifilament fibers of suture was made fromby using electrospinning method. The results of tensile tests showed that the five samples were in the optimal range, 4.62 MPa. The swelling test showed the percentage was less than 20% indicating that no swelling occurred in the wound area. The cytotoxicity assay using MTT method showed the percentage of the living cells reaching up to 90% in all samples.From the degradation test, the optimal value is showed the degradation rate of 89% at 60 days of absorption period. The antibacterial test results using *Staphylococcus aureus* are obtained the inhibition zone in range of 0,225-1,895 mm indicating that sample had inhibited the bacterial growth. Based on the characterization, PLGA collagen *Citrus paradisi* extract has potency as antibacterial absorbable surgical suture.

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Introduction

A surgical suture is one of the most important parts in wound healing process after surgery. Numerous accident cases in Indonesia have caused injuries to 117 million people annually as reported byIndonesian Central Bureau of Statistics in 2013.[1] Some of these injuries require surgical sutures. At present, many surgical sutures use a material which is nonabsorbable by the body. A surgical suture acts as a foreign matter that binds human body tissues together in healing process. Several months following the healing process, the removal of the nonabsorbable surgical suture must be done. One of the side effects of stitching wound with non absorbable material is that it can carry microorganism into the wound which may be pathogenic,i.e. it will inhibit tissue healing; and even lead to patient morbidity and mortality.[2]

The previous studies on surgical sutures indicate that stitching wounds using non absorbable polimer, *polypropylene*, still has shortcomings.The retrieval of the suture should be done shortly within 5-10 days before the healing process is completed.[3] However, asa surgical suture, it should be easily absorbed with little or no rejection from the tissues within the required duration. Its main purpose is to reduce tension of the injury.[4]

As a result, some scientists have developed the manufacturing of surgical sutures which are absorbable by the body after the healing process is completed.[5] Absorbable sutures have a slighter risk against the reaction of body rejection, but they are characteristically strong, flexible and able to dissolve rapidly in the body. Moreover, the retrieval of the suturesis no longer required

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after the healing process is completed. Thus, it is time-efficient and cost-effective.[4]

Hence, it is necessary to make a modification to improve the quality of absorbable sutures, so that they have the ability to degrade timely, they do not cause reaction against the tissues, and they have good mechanical strength and the ability to inhibit bacterial growth. Collagen is a protein that has biocompatible and biodegradable traits and has the ability to stimulate the growth of damaged tissues.[6] Collagen alone cannot be used directly because of its amorphous structure that affects the mechanical strength, so a synthetic polymer is required to increase its mechanical strength.This synthetic polymer is called *Poly (lactide-co-glycolyc acid)* or PLGA.PLGA suture has proven its excellent performance as a suture in wound healing; valuable as a polymer subtraction in combining biological materials andchemical drugs implanted in the tissues. With its tensile modulus value and long degradation, the tensile strength of the PLGA yarn is reduced to 28 % of 100 % from the initial time during 4 weeks.[7]

However, the blending of the two materials is not sufficient to improve the quality of the absorbable suture.Therefore, in this study, the addition of a natural polymer as an antibacterial action from *Citrus paradisi* is required; wherein the content of *Citrus paradisi*, namely, flavonoid has an active role in tissue response against bacteria. Many plants have been investigated for their beneficial use in different purposes.[8]

Several characterizations have been conducted in order to confirm the quality of the generated material, comprising tensile strength test to determine the strength of the material when it is given a certain load[9]; cytotoxicity test with MTT assay method to determine the level of the toxicity material when it is implanted into the body[10]; degradation test using the *Phosphate Buffer Saline* (PBS) to determine the speed of the degradation material in the body fluid simulation[11]; and the swelling degreeas well as the antibacterial test with clear zone method using *Staphylococcus aureus* bacteria to determine the resistance of gram positive bacteria against the material.[12]

Materials and methods

The Poly (lactide-co-glycolyc acid) PLGA) material used in this study was obtained from Sigma Aldrich products with a ratio of lactide to glycolide90 : 10, and with amolecular weight of 66.000-107.000 g/mol. The collagen was derived from cow bones obtained from PasarJumat National Nuclear Energy Agency, South Jakarta. The Citrus paradisi extract with ethanol was synthesized in the Faculty of Pharmacy, UniversitasAirlangga. The Chloroform and DMF were used as solvents, pro analysis materials from Merck.

The preparationfor the samples began by dissolving the PLGA (20% (w/v)) into the chloroform and DMF. The PLGA was dissolved with a magnetic stirrer until it became homogenous for ±2 hours. A total of 0.04 gram type 1 collagen was added to the PLGA solution, and then the stirring was performed for ±1 hour to dissolve the collagen. To preparethe PLGA-collagen, *Citrus paradisi* extract solution was added based on concentration variation (0,35 mg ; 0,47 mg ; 0,56 mg ; 0,7 mg). The solution was stirred by using amagnetic stirrer until it was homogeneous. The electrospinning method was used in the manufacturing of the absorbable suture candidates. A voltage of 16 kV was used to obtain good fibers. The syringe used was a 5 ml glass syringe which was filled with the sample solution at a speed of 0,25 ml/hour.

Tensile Test

Mechanical test is done by cutting rectangular sample by measuring the length and width of the specimen and the specimen thickness gauge with the micrometer screw.Membrane attributed the tip on a test and the burden of towing unit mounted on the burden of newton. Sample drawn with certain speed until breaking up. Parameter measured tensile strain (extension) and tensile stress at maximum load so it can determine the value of Modulus Young.[9]

Swelling Test

The swelling test is done to know the ratio swelling which calculated as the ratio of sample wet weight at different times and weight before PBS addition in temperature 37 ºC for 24 hours. Wet weight is calculated for several times and dried by using sponge filter paper.

Cytotoxicity Test

Cytotoxicity test was done in MTT Assay method.Then the optical density were measured using Elisa Reader and the percentage of living cells was calculated using Equation (1).

$$\text{Procentage of Living Cell (\%)} = \frac{\text{OD Treatment} - \text{OD Media Control}}{\text{OD Cell Control} - \text{OD Media Control}}$$

(Equation 1)

The parameters are value of optical densitycontrolcell [9] wherein :

Treatment OD = value of sample optical density after treatment

Cell Control OD = value of cell control optical density

Media Control OD = value of media control optical density

Living Cell % = percentage of cell numbers after treatment

Antibacterial Test

1 ose S. aureus is suspended into 9 ml TrypticaseSoy Agar(TSA) until it was homogeneous to be further bred in an incubator 60°C for 24 hours. Agar 2 % are set as nutrient for bacteria. Amount of 100 µl S.aureus and 20 mlnutrient is poured into petri dish and incubated in room temperature for 30 minutes.The culture of bacteria is made in 5 petri dish such as 1 for control and 4 others for sample based on the variation composition of Citrus paradisi extract. After incubation for 24 hours observation of inhibition zone are made. This inhibition zone used for the parameter of antibacterial value.

Degradation Test

2.5 grams PBS is dissolved in 250 ml aquabidest.Samples are immersed in solution during eight weeks while the alterations on day 0, day 10, and day 60 are noticed and noted as the dependent variable.

Result and Discussion

This study results in several samples containing PLGA-collagen compositions added with Citrus paradisi extract within ratio 1: 0.35; 1: 0.47; 1: 0.56; 1: 0.7; and controlled without the addition of Citrus paradisi extract.

Tensile Test Results

The tensile strength test or the determinations of the mechanical traits were conducted prior to the fiber manufacturing process by cutting the samples according to the rod shape required by the tensile test specimen forms. Instron was used as the tensile test equipment. The samples were pulled at a certain speed until they were broken off. After repeating the tests three times, the samples with a variety of PLGA-collagen, *Citrus paradisi* extract, were obtained with an optimal result (as shown in Figure 1), 1 : 0,7 and with Modulus Young value of 4.62 MPa which was in accordance with the value required for an absorbable suture in the abdomen.[9]

This is affected by phenol. Phenol is an organic compound commonly called carbolic acid and is a crystalline substance that has a distinctive, colorless odor, and when added with other polymers will be plasticizable.[13]

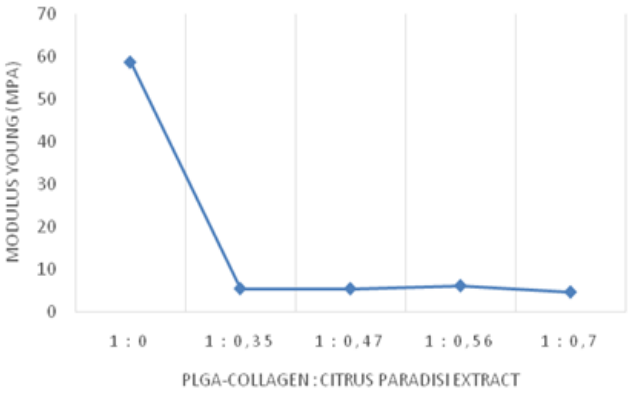

Figure 1. Tensile Strength Test Result.

Swelling Test

Excellent surgical suture has a high molecular density with a swelling degree of <50%; so it is difficult to be penetrated by the intercellular fluid.[14]As shown in Figure 2, the results obtained after weighing the five samples showed that the swelling degree value was <20%; indicating that the five samples were not able to absorb the body fluid; therefore, they would not cause swelling in the wounded area. But this result continued more than 24 hours soaking in PBS and showed that the swelling degree slowly increased within periodic time along with wound healing process until 60 days.

Figure 2. Diagram of Rod for Swelling Degree Test Results.

Swelling Degree Test

Cytotoxicity test level of the material was performedusing the MTT Assay method. Initially, the samples were incubated in the (Baby Hamster Kidney) BHK-21 cells in the Eagle's medium for 24 hours using the MTT to determine the percentage of living cells by establishing the wavelength absorbance using the ELISA Reader. If the percentage of the living cells was more than 50%, the material was then declared as non-toxic.[9] The results of the cytotoxicity test with MTT method showed that the five samples produced the percentage of living cells that reached up to 90%, as shown in Figure 3; hence, the material is not toxic and is safe to be used in the body.

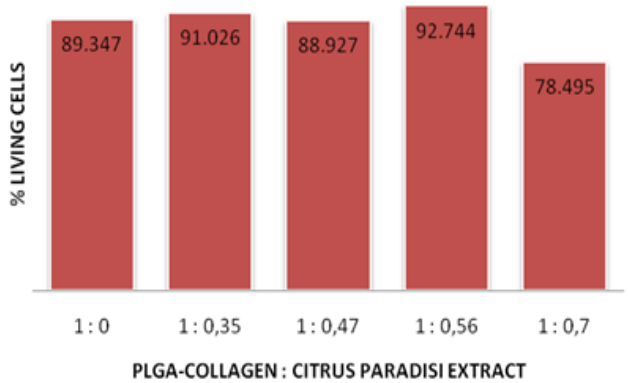

Figure 3. Cytotoxicity Test Result.

Antibacterial Test Result

The testing of anti-bacteria using *Staphylococcusaureus* bacteria was repeated 4 times. The media was made using *Trypticase Soy Agar* (TSA) as the nutrients for bacterial cultures. The incubation process was conducted 24 hours at the temperature of 37°C. The anti-bacteria activities of the *Citrus paradisi* extract were recognized from the flavonoids and phenolic compounds. The flavonoids and phenolic compounds were known as anti-bacterial agents which inhibited the growth of *Staphylococcus aureus* bacteria.[15] Based on the test results in Figure 4, the PLGA-collagen samples with the additional variety of *Citrus paradisi* extract were recognized as antibacterial as seen from the clear zone area all over the sample disks with a 0.225 until 1.895 mm diameter. The optimal sample was occupied by the sample with a variety of 1: 0.7 and a clear zone diameter of 1.895 mm. Thus, the higher the concentration of the test, the larger the diameter of the clear zone.

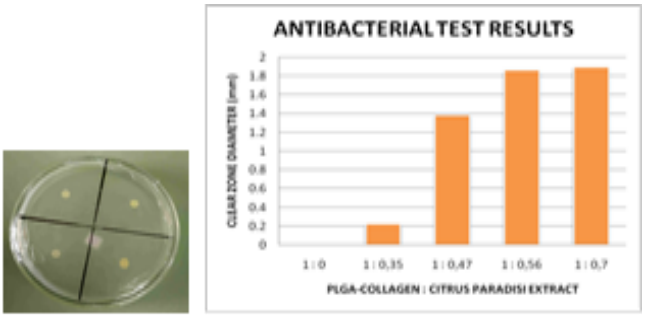

Figure 3. (a) Antibacterial Test (b) Become Antibacterial Test Result.

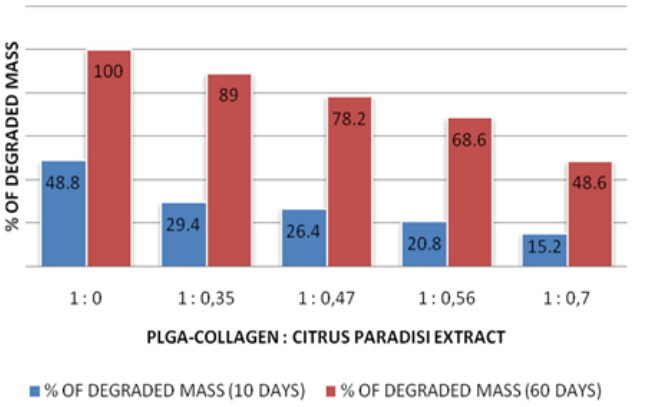

Figure 5. Diagram of Rod for Degradation Test Results.

Degradation Test Result

The degradation test was carried out using the *Phospate Buffer Saline* (PBS) as the body fluid simulated. The surgical suture is needed until skin itself is able to withstand the 80% normal strength at 60 days after the injury.[11] Based on the test results, the five samples showed that the degraded mass reached up to 89% at aspeed of 60 days of absorption, as

shown in Figure 5. This decrease in degradation rate is due to the presence of phenol compounds possessed by Citrus paradisi extract, water soluble compounds and crystalline form. Associated with higher crystallinity and higher crystal size will slow down the degradation process. From this results showed that the degradation process does not occur spontaneously but rather slowly.[13]

Conclusions

The combination of PLGA-collagen with *Citrus paradisi* extract antibacterial action is a potential candidate for absorbable suture and it is proven antibacterial based on the results of the tensile strength test, the swelling test, the cytotoxicity test, the antibacterial test and the degradation test.

Acknowledgements

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