IN VITRO CHARACTERIZATION OF POLY-GLYCOLYC LACTIC-CO ACID (PLGA) – COLLAGEN BASED ON RED SNAPPER FISH SCALES (*Lutjanus Sp.*) COATING CHITOSAN AS DURA MATER ARTIFICIAL CANDIDATE

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ABSTRACT

Head trauma is the third cause of deaths with a high rank, causing severe head injury for 25.5%-54.9%. This study has been conducted by making a replacement layer of the brain (dura) to overcome the dural defect's impact by utilizing waste fish scales red snapper (Lutjanus Sp.). Synthesis brain membranes lining processed by casting method with each various chitosan coating concentrations of 1%, 1.5%, and 2%, then dried using vacuum dry. The samples were then characterized by tensile tests, FTIR, SEM, and MTT Assay. FTIR test results showed that red snipper scales could produce collagen powder at amide A group with stretching of –NH functional group, amide B group, has stretching of CH2 asymmetry, amide I area, amide II, and amide III area, which show –NH bonding. Tensile test results showed that the combination of PLGA-Collagen Chitosan Coating 2% produced the highest tensile strength is 4.8 MPa, which met human dura mater strength standards. MTT Assay results showed that the dural membrane produced no toxic seen from living cells reached 98.32%. Poly - Glycolic Lactic - Co Acid (PLGA) - collagen coating chitosan-based on red snapper fish scales (Lutjanus Sp.) composites has potency dura mater, artificial candidate, due to the chemistry, biological, and physical characteristics.

***Keywords**: Brain; dura mater artificial; red snapper scale; collagen; chitosan*

ABSTRAK

Trauma kepala merupakan penyebab kematian ketiga yang memiliki derajat tinggi yang dapat menyebabkan cedera kepala berat sebesar 25,5% -54,9%. Penelitian ini dilakukan dengan cara membuat lapisan pengganti otak (dura) untuk mengatasi dampak cacat dural dengan memanfaatkan limbah sisik ikan kakap merah (Lutjanus Sp.). Sintesis lapisan membran otak diproses dengan metode pengecoran dengan variasi konsentrasi masing-masing lapisan kitosan 1%, 1,5%, dan 2% kemudian dikeringkan menggunakan vacuum dry. Sampel kemudian dikarakterisasi dengan uji tarik, FTIR, SEM dan MTT Assay. Hasil uji FTIR menunjukkan bahwa sisik merah dapat menghasilkan serbuk kolagen pada gugus amida A dengan regangan gugus fungsi –NH, gugus amida B memiliki regangan asimetri CH2, luas amida I, amida II dan daerah amida III yang menunjukkan ikatan –NH. Hasil uji tarik menunjukkan bahwa kombinasi antara PLGA-Collagen Chitosan Coating 2% menghasilkan kekuatan tarik tertinggi yaitu 4,8 MPa yang memenuhi standar kekuatan dura mater manusia. Hasil MTT Assay menunjukkan bahwa membran dural tidak menghasilkan toksik dilihat dari sel hidup yang mencapai 98,32%. Poly - Glycolyc Lactic - Co Acid (PLGA) - kitosan penyalut kolagen berbahan dasar komposit sisik ikan kakap merah (Lutjanus Sp.) Berpotensi sebagai kandidat buatan dura mater karena sifat kimia, biologi, dan fisiknya.

***Kata kunci**: Otak; buatan dura mater; skala ikan kakap merah; kolagen; kitosan*

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INTRODUCTION

Head is a vital organ for humans. All the control centers at the brain, which is located inside the skull of the head. Dura mater is a layer under the skull that is connected with the spinal cord at lumbal T8-9. The primary function of the dura mater is protecting the section below it, eyes, and neck. Everyone certainly has many activities every day, so every time always faces the risk of being injured. This risk may include head

trauma like an accident, bad habits, and abnormality. Incorrect posture can cause pressure on the dura mater, the layer under the skull. Besides that, suddenly, crushing can make the head trauma that causes ripped of dura mater. So that, when the dura mater is ripped or faces disruption, the organ which is protected will be disturbed too. Another case is that muscle clasp or injured can make the dura mater get risk (Humphreys et al 2003).

Inert and non-resorbable materials cover the defect in dura mater but have many side effects as foreign materials. This can cause an intense inflammatory reaction, causing neovascularization with bleeding and hematoma for the long term, and nerve will get compression (Laquerriere et al 1993). Collagen is resorbable material that has been tested in various forms, such as films, membranes, and sponges (Narotam et al 1995). The presence of collagen shows that the collagen matrix wholly is absorbed in the implantation area, and the joint of the implant cannot be differentiated with the original dura mater (Barbolt et al 2001).

Snapper fish scales is garbage that has not been maximalized by society. In terms of its large, snapper scales, this superior can be used as the leading ingredient manufacture of collagen. Collagen is a polymer with a bioactive characteristic that could stimulate cell growth and has good biocompatibility (Wei-Hong 2008). The presence of collagen as a natural polymer in the composition of the dura mater is expected to eventually help the performance of synthetic polymers, which tend to be superior in mechanical strength. Poly-D, Lactic-co glycolic acid (PLGA) is a polymer that is easily absorbed with high mechanical strength capable of adjusting for the dura mater synthesis. Due to a lack of functional groups on the surface of the PLGA, these polymers must be modified with other polymers such as collagen so that biocompatibility can be improved (Wei-Hong 2008), while chitosan is used because of its antibacterial are good and can avoid the occurrence of infection and inflammation.

MATERIALS AND METHODS

The materials were Poly (Lactic-Glycolyc-co Acid)/PLGA, collagen from red snapper scales (*Lutjanus Sp.*), chitosan, glutaraldehyde, acetic acid, NaCl, NaOH, N-Hexane, Chloroform, Aquadest. The equipment was lyophilizer, freezer, magnetic Stirrer, Shimadzu FTIR 8400S, HV-500NII for tensile test, Inspect S50, FEI Corp., Japan for morphology test, and ELISA reader.

Extraction of collagen from red snapper scales (*Lutjanus Sp.*)

We collected red snapper scales and cleaned it with soap. The clean red snapper scales were dried under sunlight for two days, then submerged with acetic acid at 0.5 M, and put in the freezer. It was put in the room temperature (27ºC-30ºC) for 1-2 hours and then filtered for the solution after freezing. The product, after being filtered, was precipitate of the collagen powder. The wet collagen powder was dried to get the dry collagen powder. Analysis of the powder by FTIR was made to ensure that the product is the real of collagen powder. After we proved the collagen include in the powder, we continued the step (Pipatcharoenwong et al 2008).

Synthesis of membrane from PLGA-Collagen composite coating chitosan

As much as 0.5 grams of PLGA with 5 mL chloroform was dissolved by a magnetic stirrer for 2-3 hours, then mold the homogeny solution by casting method. Stir for about 5-7 hours of 4-gram collagen and 4% acetic acid by a magnetic stirrer. Then, it was dried by put on the oven for 8 hours (30-40ºC). Furthermore, the membrane was ready to be deep coated with the variation of chitosan concentration (1%, 1.5%, and 2% (w/v)). After that, the membrane was dried for 2 hours at 30oC, and it was ready to be characterized.

Fourier Transform Infra Red (FTIR) test

FTIR test was performed using Shimadzu FTIR 8400S. The test was aimed to analyze the functional groups by observing the absorption of infrared radiation at different wavenumbers. Samples were characterized by infrared spectroscopy of laser light reflected by the prism. FTIR testing was required to perform KBr powder. Samples to be tested were small cut sized 0.5-1 mm and then crushed together into KBr. Samples were crushed until finely shaped by pellets and placed on a stainless steel plate.

Tensile test

This test used HV-500NII Imada, Japan. The test was done by measuring the initial length (l0), width, and thickness of samples to get the value of the surface area (A) of each sample. The sample was clamped at both ends of the holder to perform tensile tests and then given a maximum 50 N load. After the sample was stretched until it reached its maximum tensile strength, the researcher measured the final state length (l1) change. The maximum force (Fmax) and the graph of each sample were displayed on the computer screen.

The tensile strength (stress) measurement and elongation (strain) were obtained using supporting data and calculated using the formula tensile strength. Following the standard measurement of the tensile strength test, sample specimens were shaped like a dumbbell. This study's specimens followed the standard ASTM D 638 type V for thin layers such as films and membranes.

Morphology test using Scanning Electron Microscopy (SEM)

Morphology test using Scanning Electron Microscopy (SEM) (Inspect S50, FEI Corp., Japan) to explore the surface structure, pore diameter, porous/non-porous layer, and the thickness of the membrane (Sanchez et al 2012). Samples were cut in millimeters (mm) and then given a coating of gold. The next step was the insertion in the SEM tool at a voltage of 20 kV. The results of the samples that had been tested were displayed on the computer.

Cytotoxicity Test

Cytotoxicity assay is a test to identify the toxicological properties that are seen from the cell viability. This test can be done using MTT assay testing methods. The MTT Assay preparation fibroblast performance of cell cultures is derived from cells Baby Hamster Kidney -21 (BHK -21).

RESULTS

The results showed that an artificial brain membrane layer was derived from the blend of PLGA-collagen coating of chitosan. After going through the manufacturing procedure, five types of samples prepared are characterized, as shown in Table 1.

Fourier Transform Infra Red (FTIR) test result

The FTIR test results from the extraction of red snapper fish scales (Lutjanus Sp.) are shown in Fig. 1. From the test results FTIR, it can be seen that the precipitate produced scales red snapper compound containing collagen. This is evidenced by the wavelengths produced in the FTIR results at a wavelength of 3299.14 to 3544.11. This is Amida A, which shows the stretching area of functional group-NH, Amide I, at a wavelength of 1650.21. There are also 1502-1597 wavelengths, which is an area of Amida II. FTIR test results of the five samples showed an interaction between integrated materials used, namely PLGA, collagen, and chitosan.

Table 1. Artificial dura mater layer sample types

Sample Types	Composition
A (Control)	PLGA
B	PLGA-Collagen
C	PLGA-Collagen coating of Chitosan 1%
D	PLGA-Collagen coating of Chitosan 1,5%
E	PLGA-Collagen coating of Chitosan 1%

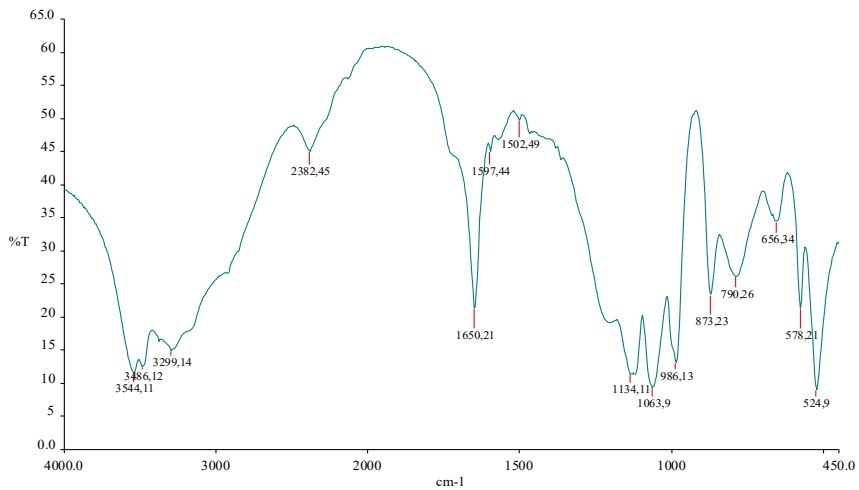

Fig. 1. FTIR spectra peak position of red snapper (*Lutjanus Sp.*) scale.

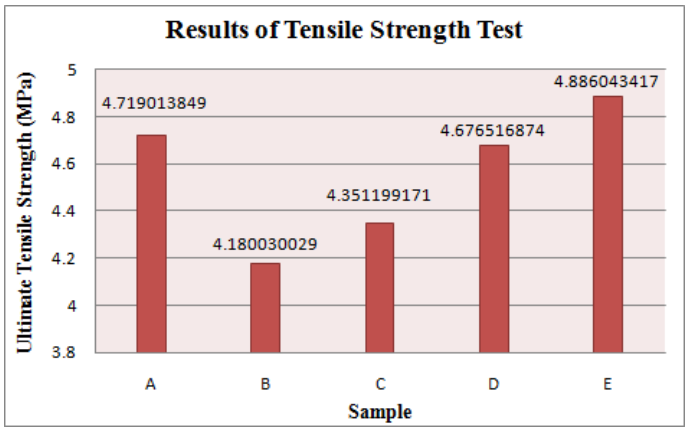

Fig. 2. Tensile strength test result.

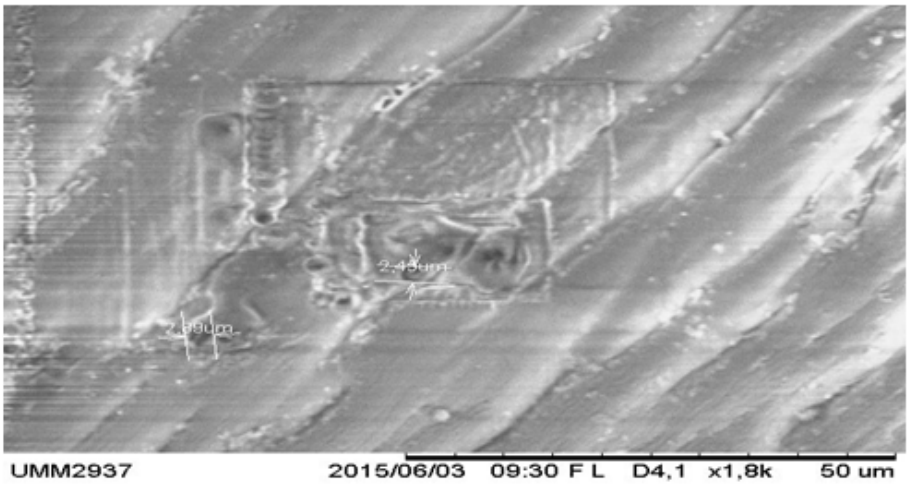

Fig. 3. The membrane surfaces composite PLGA-collagen coating chitosan 2 % structure (Sample E, magnitude 10000 times).

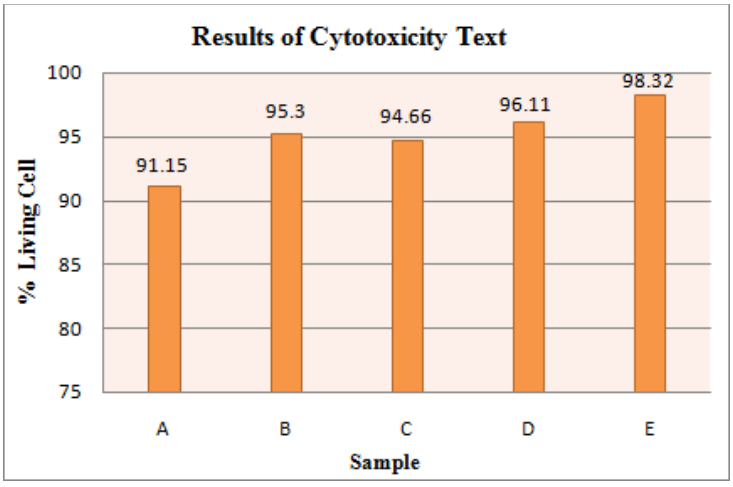

Fig. 4. Cytotoxicity test results.

Tensile test results

Sample preparation of the tensile test begins with forming dumb-bell samples, and then measured the average thickness, length early, and load selector 50N with speed 50mm/min, which have been adapted to the standards of the American Society for Testing Materials (ASTM D 1822 L), this test uses an Autograph. From the results of testing five samples, sample E produces a substantial tensile value with the highest modulus of elasticity of 4.8 MPa. This value is above the tensile strength standard of human dura mater of 2.7 Mpa (Dorian et al 2010) and is close to the value of 7.05 MPa of artificial dural membrane based on research conducted by Damien et al (2008). The tensile test results are shown in Fig. 2.

Morphology test using Scanning Electron Microscope (SEM)

A morphology test is done to explore the surface structure and pore size of the membrane. The results of SEM were smooth surface structure with pore size in the range of 2,45-2,89 µm. Sanchez et al (2012) stated that the artificial dura mater's standard pore size is 2-20 µm. The membrane from this study has been appropriate to the artificial standard, as seen in Fig. 3.

Cytotoxicity test result

The cytotoxicity status is needed as biological confirmation characteristic whether specific material is toxic or not for clinical application. The control sample had the lowest percentage of living cells compared to the other four samples. The four samples (B, C, D, E), which contained collagen, showed a high percentage of the living cell around 94, 66 % - 98, 32 %. This could be because the collagen itself as a protein is made up of amino acids, which are, in turn, built of carbon, oxygen, and hydrogen (News Medical Life Sciences 2017). Collagen can be resorbed into the body, is non-toxic, produces only a minimal immune response (even between different species), and is excellent for attachment and biological interaction with cells (Han et al 1999). The cytotoxicity results of all samples can be seen in Fig. 4.

The chart results above obtained percentage value of the overall sample of living cells of more than 60% and even reached 100%, indicating that the five samples are not toxic, then it referred to as safe for the body. This is appropriate with Róka et al's (2015) study, which stated that if the percentage of living cells > 50%, it means that the material is non-toxic.

DISCUSSION

The tensile strength of Poly - Glycolic Lactic - Co Acid (PLGA) – collagen-based on red snapper fish scales (*Lutjanus Sp.*) coating chitosan dura mater in this research is still far from the standard value of tensile strength due to concentration of chitosan. Based on the morphology test using SEM, the pore size is per the standard pore size. The cytotoxicity result showed a high percentage of the living cell. It was referred to as an excellent biocompatibility status. As natural polymers, collagen is a good material for biomedical usage. Due to its negligible immunogenicity, excellent biocompatibility, mechanical stability, and ability to be involved in all 3 phases of the wound-healing cascade (Indrani et al 2016).

CONCLUSION

Manufacture of artificial brain layer PLGA - collagen coating of chitosan has fulfilled the tensile strength and pore size dura mater in humans standards and good biocompatibility properties and potentially as a candidate Layer Artificial Brain. The candidate's best artificial brain layer was obtained on sample E with a composition of PLGA - Collagen coating chitosan 2%.

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