Pre-Clinical Research of Gelatin/Alginate Yarn for Medical Textile

Rifaida Eriningsih
Center for Textile
Bandung-West Java, Indonesia
rifaida@yahoo.com

Rini Marlina
Center for Textile
Bandung-West Java, Indonesia
rini_mrln@yahoo.com

***Abstract*- This study had prepared mixed of 75/25 gelatin/alginate yarn used as suture biomaterials. The raw materials were commercial gelatin powder of 250 Bloom gel strength and sodium alginate extracted from brown seaweed of Sargassum species. Process was done by wet spinning using spinnerets size 2000 µ. Solution consist of a mix gelatin and alginate on the viscosity of 3000 cps and pH 7, made by wet spinning method and coagulated in $CaCl_2$ solution and alcohol to produce yarn which is used as surgical sutures. Final treatment carried out with transglutaminase enzyme (TGA) and glutaraldehyde. Tests result of physical properties on the optimum condition include tensile strength of 1024 g (10 042 N), knot strength of 688.5 g (6.75 N), diameter of yarn 0.31 to 0.34 mm (No. USP = 3), and elongation of about 12%, had qualify to the requirements of U.S. Pharmacopeia 29-NF 24. Preclinical trials on mice had passed the test, i.e. no irritation occurs, can close the body tissue of their incision, wound healing clinical evidence and the yarn can be degraded in the animal tissues.**

***IndexTerms*—Gelatin/alginate yarn, wet spinning, surgical suture, transglutaminase enzyme, pre clinical test.**

I. INTRODUCTION

Biomaterials for biomedical textiles in particular, is an environmentally friendly products that began in great demand, because it can improve infection prevention, practical services, hygienic and convenient to use. Biomedical products must be non-toxic, biocompatible, does not cause allergies, does not contain harmful chemicals and non-irritating to the skin or other disorders. These products usually used as disposable products [18]. Surgical suture is one of the biomedical products serves to hold or unite the skin, internal organs, blood vessels and other tissues of the body after being cut by surgery or injury. These devices typically consist of yarn mounted on the needle. The yarn must be strong enough to hold the body tissues and flexible enough to be able to make knot easily. There are 2 types of suture: [4],

1). Dissolvable yarn by the body (disposable). This yarn can be absorbed through the enzymatic reactions in the body fluids. Absorption yarn by body tissues may occur between three days to three months depending on the type of yarn and sewn of body tissues condition.

2). Non-dissolvable yarn (non-disposable). Yarn that can not be absorbed by the body tissues and must be removed manually after the healing process. Usually not a matter of biomaterials.

Gelatin is a protein polymer derived from partial hydrolysis of collagen which is much found in skin, muscles and bones of mammals. Gelatin is a biodegradable, non-toxic and biocompatible, because it is a natural substance that contains amino acid [16], besides, easily available and inexpensive. From results of previous studies it was known that the ability of gelatin membrane could be able as therapeutic agent both for slightly or serious injuries [8,17,19]. It has also conducted research through the manufacture of gelatin fiber by wet spinning process, either 100% or gelatin mixture with alginate and PVA with variation of withdrawal process [2,9,14].

The properties of gelatin is easily deformed reversible from sol to gel form and a high water absorption, which can attract of water up to 5-10 times its original weight, but his lead is biocompatible with the human body and non-toxic [9,16], therefore, this research was conducted to get gelatin yarn for surgical sutures by mixing with alginate derived from brown seaweed extract. Mixing was intended to increase the tensile strength and knot strength, but flexible (with a particular elongation as needed) and the properties of alginate have also known was biodegradable, biocompatible and non-toxic [9,18]. For more improve the tensile strength and its degradation properties, than treated with transglutaminase enzyme as a catalyst or glutaraldehyde that can hold crosslinked with gelatin, so as to meet the requirements of U.S. Pharmacopeia 29-NF.

Transglutaminase is an enzyme that has ability to catalyze the formation of covalent bonds between free amine groups such as proteins or peptides, glutamine and lysine and gamma-carboxamide groups of protein-glutamine. Various forms of transglutaminase are found in animals (vertebrates and invertebrates), plants and microorganisms that can function in biological events such as epidermal keratinization, blood clotting, and regulation of erythrocyte membranes. Transglutaminase enzyme can be produced from microbial fermentation of Streptoverticillium mobaraense into a form of calcium-independent called as microbial transglutaminase and has been commercially available and suitable for industrial applications. Besides these enzymes may also be obtained from animal blood extraction [3].

In textile industry, the transglutaminase enzyme can be used in process of bio finishing for mixed cotton / wool fabric

that can catalyzes cross-linking and covalent bond of the two types of protein amino acids on wool fiber group such as γ - carboxyamide of peptide - bound glutamine (Glycine) and group ε - amino (Lysine) of primary amine groups. Those results of bio finishing obtained whiter wool fiber, soft and improve its strength [15].

Glutaraldehyde is dialdehyde carbon linear, soluble in water and alcohol, as well as in organic solvents. React rapidly with the amine group produce a cross linking that is stable both in thermally and chemically. Glutaraldehyde can react with functional groups of proteins, such as amine, thiol, phenol, and imidazole. lysine, tyrosine, tryptophan, phenylalanine, histidine, cysteine, proline, serine, glycine, glycylglycine, and arginine. Glutaraldehyde molecules ($HCO-(CH_2)_3-CHO$) is relatively small, consisting of two aldehyde groups are separated by a methylene bridge. Glutaraldehyde reacts with the amino groups of gelatin, for example on lysine which produces bonding imine [5].

II. EXPERIMENTAL

Materials

Commercial Gelatin powder of 250 Bloom gel strength and meets the requirements of ISO 3735-1995 (Quality of Gelatin powder), sodium alginate extracted from brown seaweed by a method according to literature [18], coagulant $CaCl_2$ and 70% alcohol of analytical grade and distilled water. Transglutaminase enzyme (TGA) used in this study is a calcium-independent form with trade name is Activa transglutaminase and glutaraldehyde (GA) of analytical grade. Solution of Simulated Body Fluid (SBF) is a synthetic solution that has approached the composition of the ionic composition of blood plasma purchased from Agricultural Institute of Bogor (Indonesia).

Experimental procedure

Solution of sodium alginate was prepared by extracted from brown seaweed (Sargassum species) got from Garut Pemeungpeuk, Indonesia, and solution of gelatin powder in distilled water. The two solutions was mixed in optimum comparison of gelatin and alginate according to the results of previous study, that was gelatin : alginate = 75 : 25, at 3000 cps viscosity [13]. Process of making yarn from those polymer solutions by means of wet spinning Laboratory scale with spinnerets 2000 μ, than was coagulated in the second stages, the first stage was coagulated in solution of 10 wt. % $CaCl_2$ to tie the alginate part and the second stage in solution of 70 wt. % alcohol to bind the gelatin. The last treatment was impregnated in transglutaminase enzyme (TGA) and glutaraldehyde (GA) for increasing its strength. Finally the yarn was sterilizes by ionizer instrument.

Viscosity of Solution

The viscosity of the gelatin solution by treatment with TGA and GA were tested to know its effect of the cross linking molecule of gelatin that was showed by increasing its in viscosity or molecular weight [3,15]. The sample was tested with a Brookfield viscosity tester. Early experiments carried out on gelatin solution at the same concentrations and at neutral pH, than was added TGA and GA at various concentration of 2% - 10%, and 0.2% - 1%, respectively. So as to estimate their effects on the increasing in tensile strength of the yarn made of gelatin / alginate. The viscosity were presented in Figure 1.

Characterization of Yarn

Physical properties of yarn included tensile strength, knot strength and elongation were tested with yarn tensile tester (Statimat) according to ASTM D 5034. From these results it can be known the optimum concentration of TGA or GA to meet the requirements of U.S. Pharmacopeia 29-NF 24. The test of yarn morphology by Scanning Electron Microscope (SEM JEOL JSM -6360LA) after immersed in SBF solution for 3 days was to know its degradation and decreasing in tensile and knot strength.

Preclinical Trials

Preclinical trials conducted at 3 mice, these animals selected according to ISO 10993-2 [6]. The mice were sliced of 1 cm long and 1 cm deep on the left and right thighs according to OECD / OCDE 404 (modified) [12]. Before incision anesthesia surgery shall be performed under general anesthesia for the mice. Incision wound on the left thigh and then sewn with yarn of gelatin / alginate (in optimum conditions) and right thigh left open. Further the two wound closed sterile gauze and every day was observed the physiological irritant (irritant contact dermatitis), allergens (allergic contact dermatitis), closing the wound tissue and evidence of healing (wound healing). This preclinical trials was carried out by physician assisted pharmacist in the Pharmacologist and Toxicities laboratory of Bandung Technology Institute.

III. RESULTS AND DISCUSSION

Viscosity of gelatin solution on the addition of TGA and GA

The effect of TGA and GA to the gelatin Solution viscositywas showed in Figure 1.

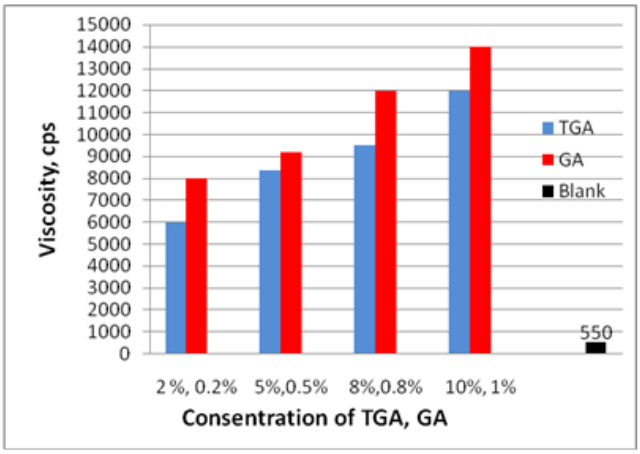


Fig. 1. Effect of TGA and GA to the Gelatin Solution Viscosity

The viscosity of Gelatin solution increases with increasing concentration of TGA and GA from 6000 cps to 12 000 cps and 8000 cps to 14,000 cps, respectively, while the viscosity of blank was 550 cps. In addition of TGA, this increasing of viscosity indicates an increase in the molecular weight of gelatin due to the influence of the TGA which catalyze amino acids in the form glycine and lysine of gelatin molecules as described in Figure 2. TGA is a single polypeptide chain has

molecular weight of 38 000, consisting 331 amino acids. It can catalyze the formation of covalent bonds between groups of two amino acids (glutamine and lysine) in protein to be a polymerization of protein with bond formation ε - (γ-glutamyl)-lysine intermolecular or intramolecular. Those formation causes more solid, viscous and the gel stability was increase. TGA stable against heat treatment, non toxic and resistant to proteolitik degradation [15].

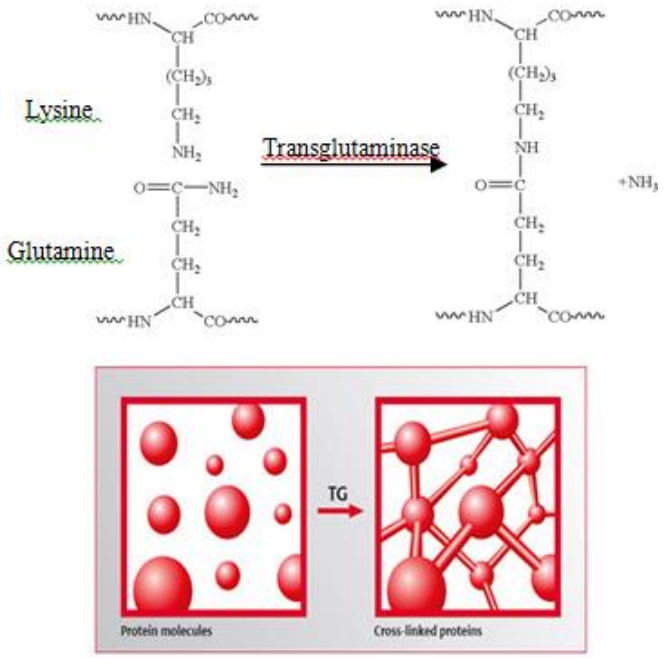


Fig. 2. TGA As Catalyzer to Form Cross Link of Protein (Glisin and Lisin)[3]

In addition of increasing concentrations of GA in the gelatin solution there was also an increasing in viscosity. Gelatin is a protein which contains amino acids consisting of Glycine-proline-hydroxyproline form a polypeptide chain. GA has a high reactivity to conduct the reaction with several functional groups in proteins, such as amine, thiol, phenol, and imidazole and amino groups such as tyrosine, Typhoon trip, and phenylalanine, histidine, cystine, proline, serine, glycine, glicilglicin, and arginine. The reaction of gelatin with GA to form cross links may occur as in Figure 3A or 3B depending on the influence of some conditions such as pH, concentration, temperature or other influences. In Figure 3A, GA reacts with the amino group of gelatin to form a redox reaction. Cross-reactions can also occur as shown in Figure 3B due to the formation of ά, ß-unsaturated aldehydes from condensation GA, thus forming a double bond [5]. The reaction looks more complex, because GA has a high reactivity to form cross-reaction, so that the viscosity is greater than the addition of TGA

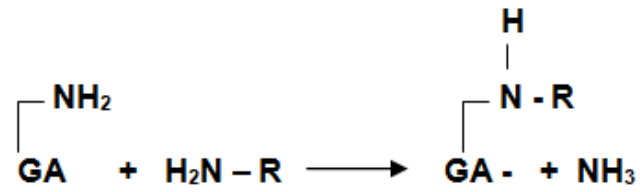


Figure 3A. Cross reaction of GA to Establish Gelatin Redox Reactions

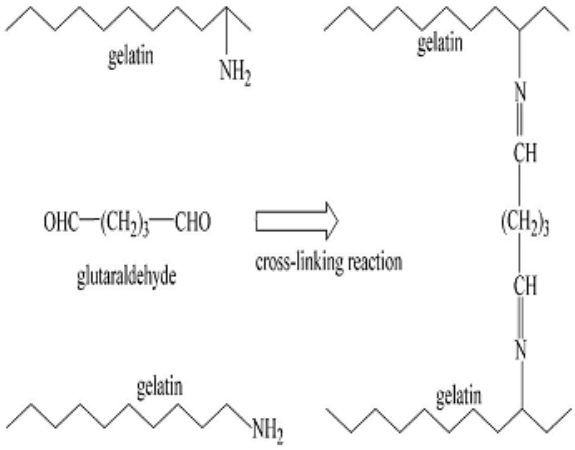


Figure3B. Mechanism Of Monomeric GA Reaction With Amine Groups On Gelatin To Form Cross-Links (DoubleBond)[12].

Gelatin/Alginate Yarn

From the experimental results of yarn made of mix gelatin / alginate in optimum condition (gelatin / alginate: 75/25) according to the results of previous research, the process is then performed with the GA and TGA. Test results of tensile strength and knot strength seen an increase as shown in Figure 4.

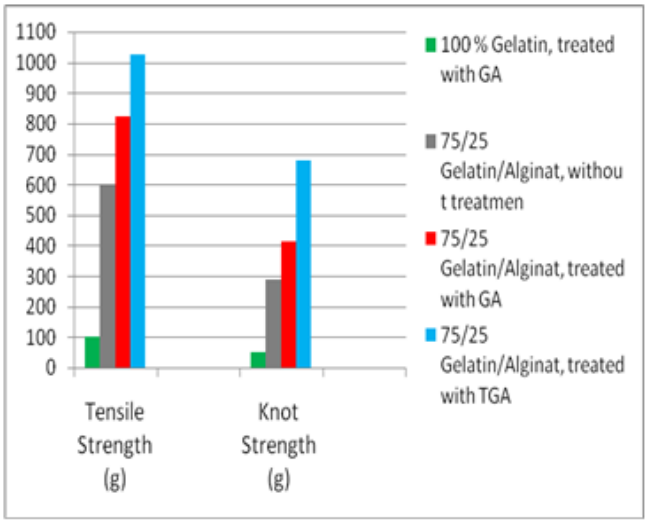


Figure 4. Tensile Strength and Knot Strength of Gelatin / Alginate Yarn

The 100 % of gelatin yarn (without alginate mixture) gives low in tensile strength and knot strength. This can be explained that gelatin can attract water up to 10 times the initial weight, easy sticky because has low in melting point (27-32°C), thus producing yarn that is less solid and easily affected by environmental conditions such as humidity. By addition of the alginate solution into gelatin will form a homogeneous mixture. Carbohydrates or polysaccharides (alginate) and

protein (gelatin) were able to synergize to form a better texture, flexible and increase its strength. The melting point of alginate is 300°C, then mix the two types of those hydrocolloid will result a yarn that is more heat stable and can increase the melting point [13]. The last treatment with GA and TGA will increase in tensile strength and knot strength and will stable in storage. It has also been proved from the test results of gelatin viscosity by treated with TGA or GA to be transformed into a thick gel form with increasing viscosity. Test results of tensile strength and knot strength of yarn that use of GA gives higher than TGA. The use of GA excess (> 0.8 cc/l) will cause toxin due to residual aldehyde which does not bind to the protein [1]. To prevent that, the use of GA have chosen at the safe concentrations of 0.5 %, but its strength test results is less than optimal.

From the experiments mentioned above, it can be selected the use of 10 wt. % of TGA as a catalyst to form cross linking of gelatin molecules, that provides optimum tensile strength of 1024 g (10 042 N) and knot strength of 688 g (6.75 N). For more than 10 wt. % of TGA the viscosity of solution can not be detected. The important thing is the TGA is a natural substance and not be toxin.

The elongation of yarn greatly affect to its knot strength. The use of GA causes the yarn rather brittle than with TGA, while the yarn without GA or TGA treatment its elongation is low (3.8 %) and more brittle. The elongation of gelatin/alginate yarn on the use of TGA was 11,98 % and more elastic as shown in Figure 5.

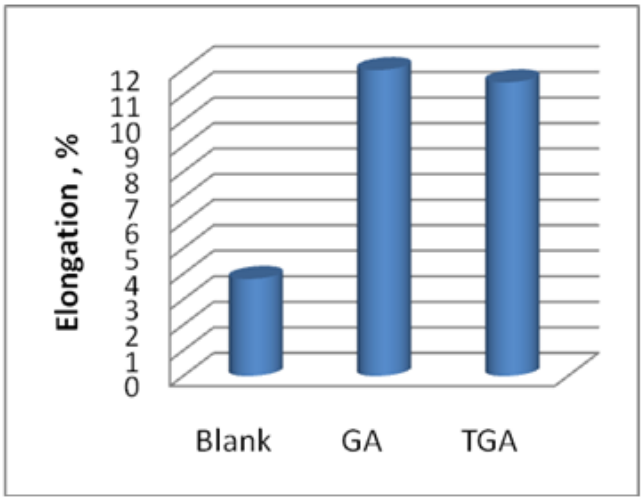


Figure 5. The Elongation of Gelatin / Alginate Yarn

Based on test results above, the physical properties of yarn at optimum conditions of gelatin / alginate yarn (75/25) with 10 % of TGA treatment can meet the requirements of U.S. Pharmacopeia 29-NF 24 for the yarn diameter of 0.310 to 0.340 mm or equal to surgical suture no. 3 [1,20].

TABLE I. THE REQUIREMENTS OF US PHARMACOPEIA 29-NF 24

	Results of this study	**US Pharmacopeia 29-NF 24**
Yarn diameter (mm)	(0.31 – 0.34) mm	No.3 (0.300-0.339) mm
Tensile strength	1024 g	1.05 kg
Knot strength	688.5 g	(0.68- 1.25) kgf

IV. DEGRADATION TEST

From test results of morphological structure of the gelatin / alginate yarn after degradation test by immersed in SBF solution for 3 days [10], noted that yarn without treatment with TGA ((Figure 6 A and A-1) is more easily degraded shown on the cross section and elongated structure that looks eroded and not compact. Morphological changes is marked by hydrolysis of chemical bonds in the polymer chain which can produce the blanks or cracks. The first hydrolysis occurs in amorphous regions, as these areas more accessible to water molecules and enzymes. After treatment with TGA (Figure 7 B and B-1), the morphologic structure of yarn looks more regular, compact and its crystalline structure is still visible.

This test also proved its strength from the test results shown in Figure 6, which is comparable with decreasing of its strength.

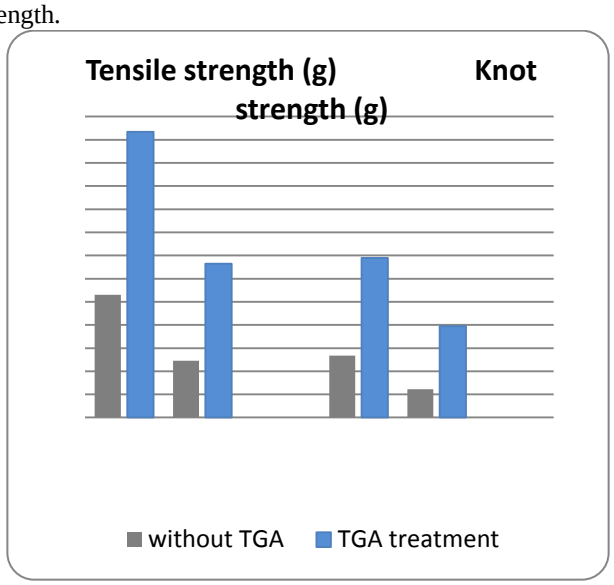


Figure 6. Tensile strength after immersion in SBF solution

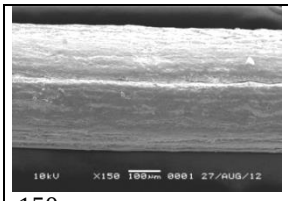
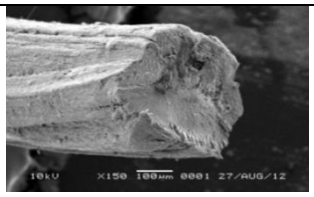
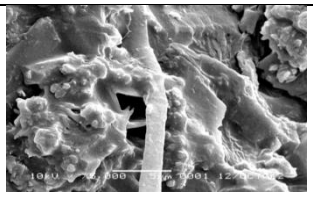
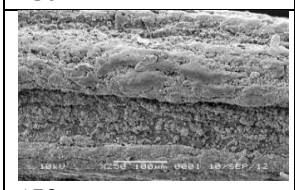
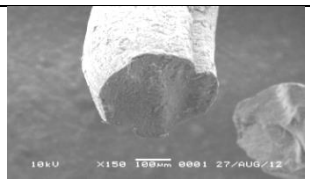
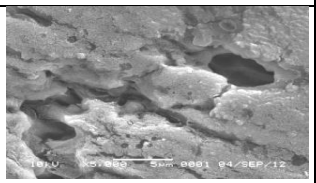
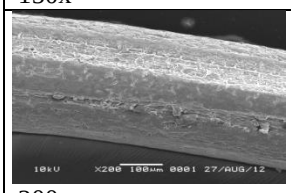
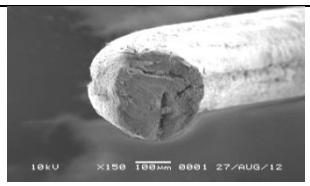
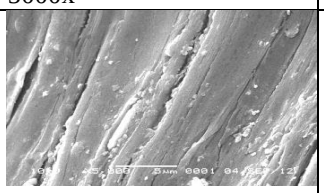
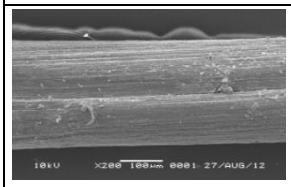
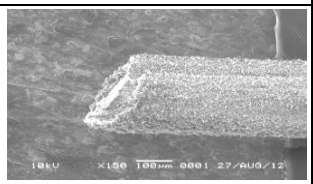
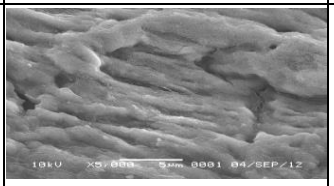
 150x	 150x	 5000x	A : gelatin/alginate yarn without treatment
 150x	 150x	 5000x	A-1: yarn A after degradation test
 200x	 150x	 5000x	B : gelatin/alginate yarn treatment with TGA
 200x	 150x	 5000x	B-1 : yarn B after degradation test

Figure 7. Degradation of Gelatin/Alginate Yarns After Treatment in SBF

From those data shown that the decrease in tensile strength and knot strength after immersion in SBF solution as presented in Table 2 indicate that the yarn still meet the requirements that a maximum of 50 % decrease or for implantation of *in vitro* pre degraded material up to 50 % weight loss or 50 % loss of mechanical strength [7].

TABLE II. DECREASE INTENSILE AND KNOT STRENGTHAFTERDEGRADATIONTEST

Decrease inTensile and knot Strength		
	without TGA	TGA treatment
Tensile Strength	60%	42.2 5%
Knot Strength	59.4%	42.67%

V. PRE-CLINICAL TESTING

Preclinical trials conducted on the first day by making an incision in both left and right thighs of mice as shown in Figure 8 day 1. On the same day the wound on the left thigh of mice sewn with yarn of gelatin / alginate, but on the right thigh left open (blank). Next to the two wounds were closed with a sterile gauze bandage. On the second day the observation was done by opening gauze pads and it still looks bloody wound that has not dried. Observations on the third day was still looking wound, but it was a bit dry and yarn still seen.

Day 4 showed the wound began to dry, the yarn a little bit seen and start to be degraded. Day 5 shown that the wound was dry, stitches and yarnrarely seen. Observations on Day 6, the wound is dry and healed, scar just stay, no infection or irritation and suture or yarn just stay a little bit (degraded). For the other wound without suturing (on the right thigh of mice), on day 6 wound remains open, around the wound reddened and festering. Furthermore on the day 7, which was not sutured incision showed not festering anymore after given treatment.

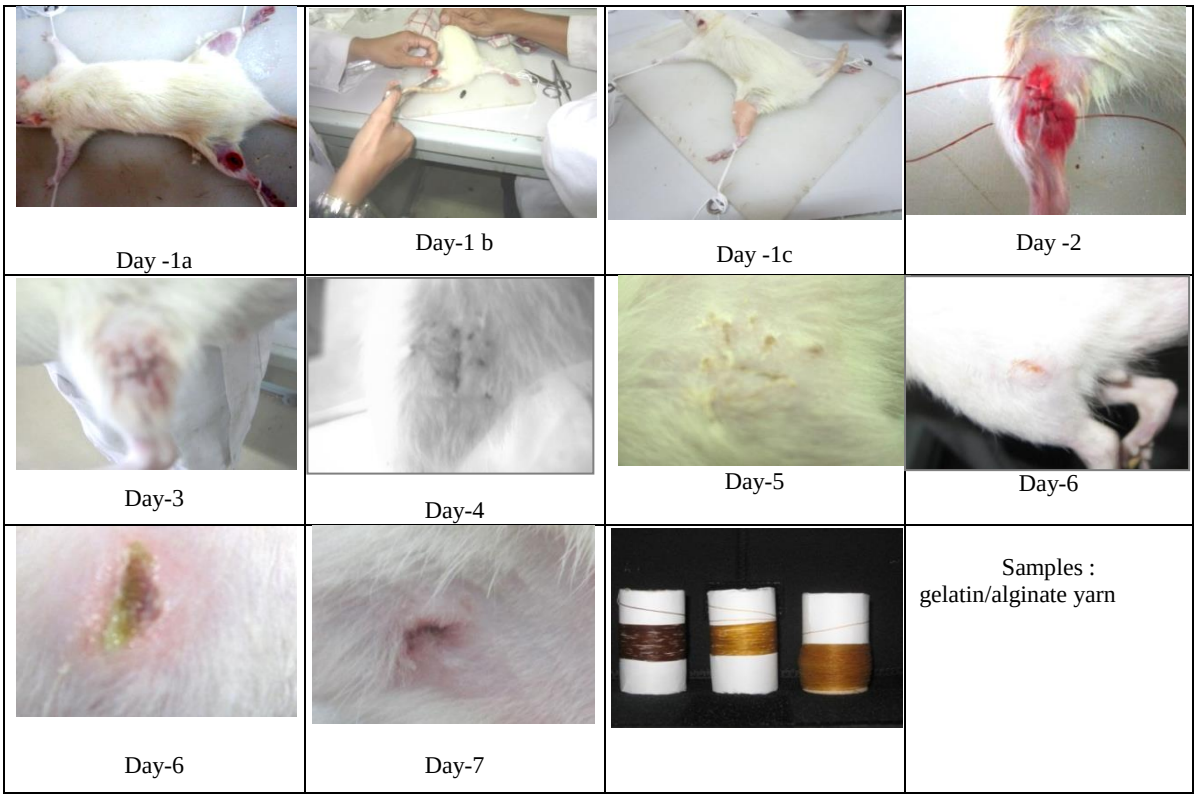


Figure 8. Pre-clinicalTestResultsonMice

TABLE III. OBSERVATION OF THE INCISION WOUND OF MICE

Day	Incision wound on the left thigh of mice and sewn with gelatin / alginate yarn	Incision wound on the right thigh of mice without sewing (blank)
Day -1a	Incision on right and left thigh after anesthesia	-
Day -1b	Suturingthe wound on left thigh, 2stitchesand2knots. Not sewnright thigh (blank)	-
Day -1c	Closure ofthe woundwith agauzebandageandplaster (right andleftthigh)	-
Day -2	The woundis stillwet, suture seems clear	-
Day -3	Still looking scar, the yarn is still looks	-
Day -4	Woundsbegan todrythe yarnlookslittle sketchy(ranging degraded)	-
Day -5	Woundwas dry, stitchesandyarnseemed rarely	-
Day -6	Woundis dry andhealed, scarjuststay	Incision not sutured (right thigh/blank), woundremains open, roundredandfester
Day-7	-	No fester after given treatment (give medicine)

From the results of this pre clinical trial indicate that the yarn of gelatin / alginate can be used as surgical sutures for animal (pre-clinical observation in animal), there are non toxic, biodegradable and biocompatible with body tissue. of experimental animals. Those results also gave no irritant, no allergens, the yarn were capable to close the wound tissue and evidence of healing (wound healing).

VI. CONCLUSION

From the results of this study, to obtain yarn gelatin / alginate 75/25 by wet spinning process, which meets the requirements of U.S. Pharmacopeia 29-NF 24, then in the process needs to be added transglutaminase enzyme at a concentration of 10 wt.%, therefore obtained the tensile strength of 1024 g (10 042 N), knots strength of 688.5 g (6.75 N), yarn diameter 0.31 to 0.34 mm (No. USP = 3), and elongation of 12%. Pre-clinical trials conducted to prove that the yarn can be used as a suture through experiments on mice and successfully passed the test, no irritation occurs, can unite the body tissue of the incision at a certain depth, clinical evidence of wound healing and biodegradable yarn in the body tissues of experiment animals.

ACKNOWLEDGEMENTS

Addressed to Prof. Elin Yulinah Sukandar who helped in conducting pre-clinical trials in the Laboratory of Pharmacology and Toxicity ITB.

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