Biocompatible Material from Indonesian Natural Resource of Wild Silkmoth Cocoon

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Abstract. Silk can be produced by spider or insect and have prospect as biomaterial for regenerative healing in medical treatment. Silk having physical and chemical properties that support biocompatibility in the living things. In this research, silk that was obtained from Indonesia natural resource of *Attacus atlas* silkmoth was explored and then will be developed for biocompatible biomaterial. The treatment with NaOH was developed to separate the fiber from the cocoon. The obtained fiber is investigated its mechanical property by performing tensile test for single fiber. The biocompatibility testing was conducted with human cell (osteosarccoma) cultivation. The result identify that separation by using NaOH yield better better mechanical properties comparing konvenstional method with boiling in hot water. Biocompatibility testing indicate that the the fiber having good biocompatibility.

Keywords: Silk, wild, Attacus atlas, mechanical properties, biocompatibility

I. INTRODUCTION

Natural silk is an interesting material for application in biotechnology and biomaterial that is obtained from spiders or insect [1,2, 3]. Silk exhibit unique properties such high tensile strength with good properties of biocompatibility [4,5]. The research related with silk biomaterial commonly uses silk that is obtained from the species of domesticated *Bombyx mori*. This species was domesticated more than 4000 years ago and historically for use as material for fabrics [6].

Fiber obtained from cocoon of silk mainly contain complex fibroin which is produced by labial glands [7]. Around 10-12 μm fibroin fiber of *Bombyx mori* contain amorphous protein that is sericin which is released from cocoon by washing with weak alkali or hot water. The process of releasing fiber from cocoon is so called degumming [8]. Fine fiber that is obtained from degumming process can be used as source of fibroin. The fibroin can be dissolved with lithium salt, calcium nitrate or organic ion liquid. A liquid fibroin can be fabricated for fiber, sheet or three dimension product with pore (sponge) [9].

There species other than *Bombyx mori* dan can also produced silk with unique properties that cannot be found in the *Bombyx mori* one. For Example is *Antheraea pernyi* that have better property in cell cultivation *feline fibroblast* [10]. Also with species *Antheraea mylitta* as well, that have better property for the growth and attachment for *cardiomyocytes* from mouse postnatal [11].

Unique attachment properties of *A. mylitta* fibroin matrix is caused by content of short amino acid RGD motif in the molecule [12]**.** The tensile strength of silk obtained from silkmoth cocoon compared with silk from spiders, especially spiders from species of *Nephila* was found different [13]. Method to release the fiber from the cocoon (degumming) influent the properties of the silk [14,15].

Fig. 1. The cocoon of silkmoth of *Attacus atlas* and appearance of the silkmoth.

In this research, the cocoon from species of *Attacus atlas* silkmoth (Fig. 1) is taken as a source of silk fiber that was degummed by using NaOH solution. The fiber obtained will be tested its biocompatibility troughs growth of human cell osteosarcoma (U2OS). The tensile test is performed to discover the mechanical property of single fiber.

II. RESEARCH METHODS

The cocoons of silk-moth *Attacus atlas* were prepared from Indonesian sources. The Cocoon was boiled in).1 M of NaOH for 1 hour. The fibers obtained by using this process were cleaned with hot water. The 50 mg of fiber clean fibers were sterilized in ethanol for 12 hours at room temperature. Afterward, the samples are washed with 4 time repetition with PBS and then separate from the PBS solution with vacuum suction.

.The Fiber then was immersed for 2 hours in to the suspension of U2OS cell with media cultivation of DMEM with supplement of 10% FBS (10^5 cell per ml). The atmosphere was 5%CO_2, at temperature 37ºC and 95% humidity. The cell cultivation was conducted for 6 days. Observation of cell growth of the fiber was carried out by taking the fiber from cultivation media and cleaned twice with PBS

The tensile test was conducted by following tensile test method for single silk fiber [16,17,18]. The 5 cm length of single fiber was glued in the cardboard frame with distance 30 cm (regarded as gauge length). The tensile test was performed by using screw test stand with constant speed. The balance with resolution ± 10 mg was mounted at the bottom of the cardboard frame of the tensile test to measure the load as similar with loas cell. The testing was performed at 30ºC and 60% relative humidity.

Cell on the fiber surface was fixed with 4% formaldehyde in PBS for 20 minutes and washed twice. The cell nucleus was stained with 1 µg/ml DAPI (Sigma-Aldrich, USA) and actin microfilaments with 10 µg/ml phalloidin conjugate with TRITC in PBS solution for 20 minutes at room temperature. The observation was performed by using fluorescence microscope

III. RESULTS AND DISCUSSION

The *Attacus atlas* fiber contained with bonding material together with fibrillar material as depicted in Fig. 2. The bonding material is removed completely (Fig.3) with NaOH treatment with concentration 0.1 M. It is found the tensile strength of the fiber is higher if treated with NaOH compared the one with conventional degummed method with boiling in hot water as presented in graph in Fig.4. The comparison of the tensile strength result can be observed in Table 1 and Table II.

TABLE I
THE TENSILE STRENGTH OF *ATTACUS ATLAS* FIBER DEGUMMED WITH CONVENTIONAL TREATMENT(BOILED IN HOT WATER)

Silk type	*E* (MPa)	ε_u	σ_u (MPa)
A. atlas	298±63.42	0.17±0.020	51±14.69

TABLE II
TENSILE STRENGTH OF *ATTACUS ATLAS* FIBER DEGUMMED WITH NAOH TREATMENT

Silk type	*E* (MPa)	ε_u	σ_u (MPa)
A. atlas	604±36.65	0.17±0.012	101±13.28

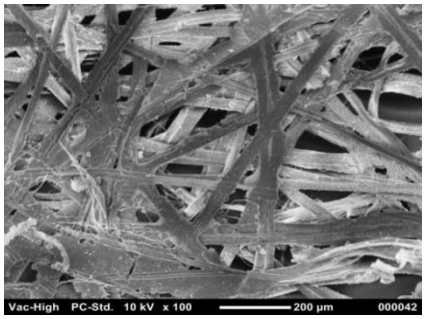

Fig 2. Scanning Electron Micrograph (SEM) of *Attacus atlas* cocoon before degumming with NaOH (raw material)

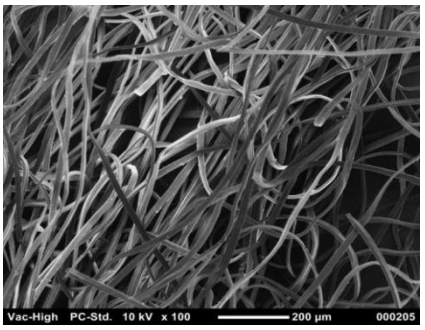

Fig. 3. Scanning Electron Micrograph (SEM) of fibner obtained from cocoon of *Attacus atlas* after degumming with NaOH

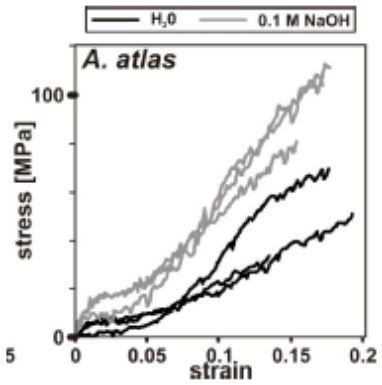

Fig. 4. Tensile test result. It is found that degumming with NaOH is found having higher tensile strength compared with the one with conventional degumming method of boiling in hot water

Biocompatibility testing of *Attacus atlas* fiber with 0.1M NaOH treatment indicate that human cell osteosarcoma cell line (U2OS) able to attach and grow on the surface of the fiber in the next 6 days. The fiber is fully covered with U2OS cell that indicate the fiber having good biocompatibility (Fig.5)

It was discovered in the previous research that *Attacus atlas* and other species from family *Saturniidae* containing high calcium oxalate [19]. In this research with 0.1 NaOH the mass of the fiber can be reduced up to 20% from initial condition. This data indicates that the degumming process with 0.1 M NaOH not only can

eliminate the sericin but also the tanning process to occur that influents the cross linking of the protein [20,21,22].

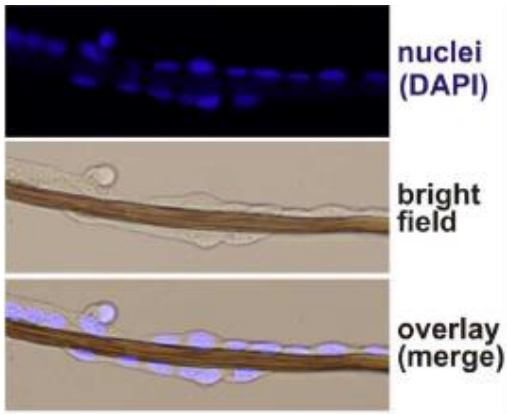

Fig. 5. Excellent biocompatible fiber is found after NaOH treatment of Atacus atlas fiber. The fiber is found covered entirely with human cell of U2OS

IV. CONCLUSION

It can be concluded that NaOH treatment is suitable method for degumming of *Attacus atlas* cocoon. The precise concentration is found at 0.1 M NaOH. With degumming method, the fiber is found having excellent biocompatible to human cell. The fiber also is found having better tensile strength compared with if degummed with conventional way by boiling in hot water.

ACKNOWLEDGMENT

This research was supported by Scheme of *Penelitian Kerjasama Luar Negri* PNBP Udayan University 2016 together with Czech Science Foundation Project P108/12/G108.

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