New Route in Degumming of Bombyx Mory Silkworm Cocon for Biomaterial

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***Abstract*—This research focused on silkworm cocoon of *Bombyx mori* grown in Indonesia. In this study the degumming process to eliminate the sericin from the fiber was explored and the result is a separated fiber that is tested for its biocompatibility. The silk can be prepared by degumming method of boiling in 0.01 M NaOH for 1 hour. Observation under microsccope indicate that The human osteosarcoma cell line (U2OS) able to attach and grow during following two days. This is an indication that the fiber having good biocompatibility by degumming process that is introduced in this report.**

***Index Terms*—bombyx mori, silkworm, cocoon, degumming, biocompatibility**

I. INTRODUCTION

Some *Lepidoptera* larvae such as silkworm, spiders produce fibers that are recognized as a silk [1]. *Bombyx mori* (Fig. 1) is the most famous silk and have been domesticated from ancient time. The mulberry leave (Fig. 2) is the preferred food for the *Bombyx mori* larvae. The silkworm then will produce cocoon as depicted in Fig 3. The appearance of *Bombyx mori* silk moth can be seen in Fig. 4.

Beside well-known in textile production, *Bombyx mori* of silkworm silk has been used as medical suture. Sutures require the characteristics such as: Free from infection, non-irritant, possible to be metabolized once its repair function has been completed, the stiffness is change over time, the ability to conform to the current stage of wound repair, Knot strength, tensile strength to match the clinical repair [1]. From just for medical suture, silk then become point of interest by the researcher to provide biomaterial for other purpose.

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It was proofed that *Bombyx mori* is suitable for 3-D scaffolding material which is make possible for the cell spread along the fiber and after that covered the entire surface and grow to fill the gap to form the structure of the tissue [2]. Silk also possible to be used as implants for the healing of critical size of bone defects which demonstrate the feasibility of silk-based implants with engineered bone for the regeneration of bone tissues with a mechanically stable and durable option [3].

Figure 1. Silkworm larvae of *Bombyx mori*

Figure 2. Mulberry tree

Two major proteins contains in the silk that are sericin (which is the coat and encased of the fiber) that hold

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fibroin fiber together to form cocoon case in protect the worm inside [1]. Sericin is antigenic gum-like protein surrounding the fibers and fibroin is the core filaments of silk comprised of b-sheet crystal regions and semi-crystalline regions which is responsible for silk's elasticity [1].

The sericin induces hypersensitivity in patients, causing a Type I allergic reaction. Exposure to silk debris may sensitize patients to silk causing adverse allergic reactions when silk is used as a suture material. Sericin identified as the antigenic agent of silk and should be removed through process of degumming [1]

Before further processing for biomaterial, the silk usually should be degummed first in order to fine fiber will be obtained. It is common for *Bombyx morri* cocoon to be degummed by boiling in hot water bath. samples were placed in beaker, and sufficient distilled water was added to completely immerse the sample. The beaker was heated in a hot water bath, and the samples were then washed with heated distilled water [4]. But in practice this technique will not eliminate the sericin in total as can be seen in Fig. 5

Figure 3. Silkworm cocoon of *Bombyx mori*

Figure 4. Silkworm moth *Bombyx mori*

Other protocols for degumming of the silk also already established such as by using Na_2CO_3. Cocoons from *Bombyx mori* were boiled for 1 h in an aqueous solution Na_2CO_3, and rinsed with water to extract sericin and other contaminating [3]. Other modification from this technique is

Dried *Bombyx mori* silk cocoons were cut into small pieces and treated with boiling aqueous solution of sodium carbonate with stirring. The mass was repeatedly washed with distilled water to remove the sericine protein and dried in hot air oven [5]. Usualy after this process it is continued by dissolved in LiBr [6] or formic acid [7]. It was also informed that degumming also possible by using urea urea [8]. All degumming methods above mentioned having advantages and disadvantages that the reader can find it in the reference cited.

Degumming is also possible for removal of sericin by using enzymes protease and lipase Enzyme degumming involves the proteolytic degradation of sericin, using the specific proteins with minimum effect on fibroin. When the substrate molecule fits into the active sites of the enzyme's molecular structure to form an enzyme substrate complex which is yields an end product and the original enzyme molecule is reproduced. Enzymes treatment operates under low temperatures and mild conditions which reduce the energy consumption. The disadvantage of this method is lower performance of enzyme degummed silk including difficult to handle and high cost. This condition limited the application of enzymes on the silk industry [8]

The action of organic acids is generally less aggressive than that of an action by alkali solution. Acidic agents (tartaric acid or citric acid) for degumming was approved for physical property enhancement. The action of organic acids is generally less aggressive than that of an action by alkali solution. the high performance on degumming is achieved by tartaric acid in terms of sericin removal efficiency and of intrinsic physico-mechanical characteristics of silk fibers. In the case of critic acid for degumming yield dry and wet resiliency of silk which was remarkably increased with citric acid treatment but acid causes the damage on the fibroin surface [8]

Figure 5. result by degumming in boiling water. The sericin was not totally eliminated

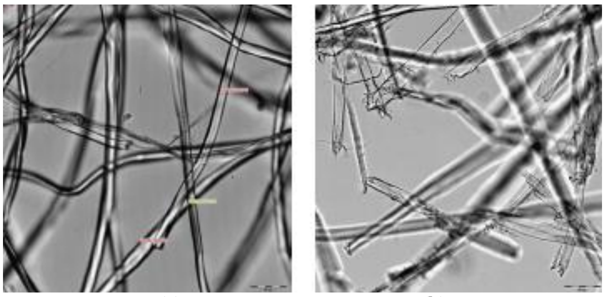
This research introduce new route in degumming of silkworm cocoon of *Bombyx mori* as complement of our previous work [9], [10], that can be used as alternative in order best choice can be achieved with limited disadvantages.

II. Experimental

Bombyx mori cocoon obtained from local collector from Indonesian source (fig.3) was incubated in water and NaOH solution (two concentration were prepared: 0.01 M and 0.1 M) and boiled for 1 hour. The samples then were shaken for about 20 seconds. The insoluble fiber then was washed in warm water (70^oC) intensively. Scanning Electron Microscope (SEM) was utilized to observe the structure of the fiber before and after degumming. The results fibers were sterilized with 70% ethanol for 1 day at room temperature. The samples were washed by PBS and suspension of human osteosarcoma cell line (U2OS) cell in Dulbecco's Modified Eagle's cultivation medium and then weere soaked (in the atmosphere (5% CO_2 at 37^oC and 95% humidity) for 2 hours. The cultivated medium was supplemented with 10% FBS. The cells were cultivated for 2 days. For observation of the cell, the sample was taken out from cultivation medium and transferred to PBS and washed. Fluorescence microscope was utilized to observe the cell growth (IX-71, Olympus Japan) as characteristic of biocompatibility.

III. Result and Discussion

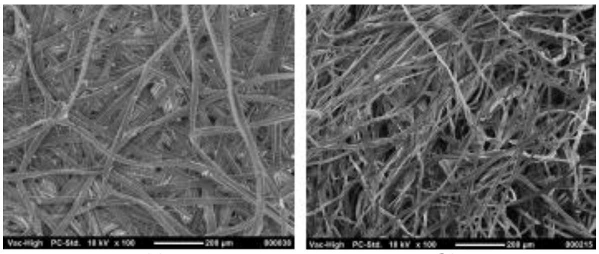
The efficient removal of the sericin coat was observed after treatment by 0.01 M NaOH as presented in Fig. 6a and Fig 7b. By using 0.1 M NaOH resulting crashed fiber which is indication of strong hydrolysis of fibers, there fore not recommended (Fig. 6b).



(a) (b)

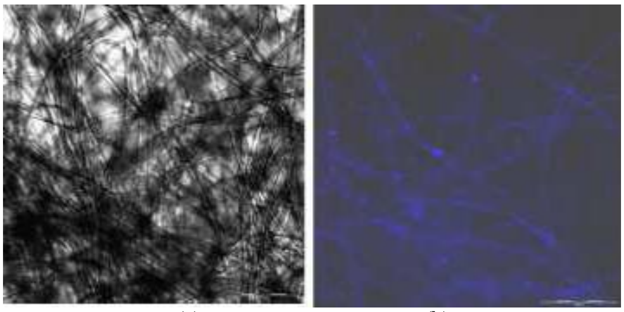
Figure 6. (a).After treatment by 0.01 M NaOH, (b). By using 0.1 M NaOH resulting crashed fiber which is indication of strong hydrolysis of fibers

It was found that the cells are able to attach and grow on *Bombyx mori* fiber released from cocoon by 0.01 M NaOH during following two days base on observation under microscope. This is an indication that the fiber having good biocompatibility (Fig. 8). Our previous report [10] also informed that deguming by using 0.01 M NaOH also resulting good deguming result to other type of cocoon (*Cricula trifenestrata*) and resulting positive indication for biocompatibility.



(a) (b)

Figure 7. Scanning Electron Micrograph (SEM) of the Bombyx mori silk fiber (a) condition before degumming as obtained from our previous publication [9]. (b) after degumming with 0.01m NaOH



(a) (b)

Figure 8. Cell growth on Bombyx mori fibers obtained by degumming of cocoon by using 0.01 M NaOH. (a) bright field. (b) After cell fixation, nuclei were stained using DAPI and analyzed by fluorescence microscopy

As it is found by using only boiling water [4] for degumming, then the sericin is not totally removed (fig. 4). Other effect is the tensile strength is found low [4]. This research should be continued to prove that by using degumming method introduced in this report will also increase the tensile strength.

Comparing with other method of degumming, as for example by using Na_2CO_3 [3], [5], formic acid [7] and urea [8], the method that is introduced in this research is also possible to be introduced to other types of silk cocoon such as from the species of *Cricula trifenestrata* [9], [10] and also Attacus atlas [9] meanwhile in our experience degumming by using Na_2CO_3, formic acid or urea are not successful for degumming both cocoon of *Cricula trifenestrata* and *Attacus atlas*. Other negative side by using Na_2CO_3 is, sericin is emulsified by the soap and finally, removed from the fibre. but, the presence of soap and alkalis in the wastewater of degumming process raise an issue of pollution. Besides, the degumming cycles of the soap ash bath is limited because of acidity of sericin hydrolysis products accumulating in the bath [8]

Other advantages of degumming method that is introduced in this research is, it can shorten the process. Other technique should initiate by boiling in water first and then continued by the next process, meanwhile in the technique which is introduced in this research is possible without initiate by boiling water. So that it can shorten the process for the benefit of production cost.

IV. Conclusion

The new route in degumming silk of *Bombyx mori* is established in this research. The silk is prepared by degumming method of boiling in 0.01 M NaOH in 1 hour. The human osteosarcoma cell line (U2OS) able to attach and grow during following two days. This is an indication that the fiber having good biocompatibility by degumming process that is introduced in this research.

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