

Indigenous Indonesian Wild Silkworm Cocoon of *Attacus atlas* as Biocompatible Film Biomaterial

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Abstract. The biocompatible film made from wild silkworm cocoon of *Attacus atlas* is introduced in this research for anticipation of demand on biocompatible film for regenerative medicine. The wild silkworm cocoon was indigenous Indonesia and was taken from its original location in Indonesia. Protocol for degumming method was obtained in this research by using treatment with NaOH solution at 0.1 M for 1 hour. The film was prepared by grinding the wet degummed fiber until pulp like state was obtained. The mixture was dropped in sequence on the hot ceramic plate with temperature around 60°C. The film thickness can be controlled precisely by using this technique. The film is soaked in alcohol for 1 day for stability testing and the result is found stable. The film is introduced into COS-1 Cell suspension with previously washed in PBS solution and put in a chamber for biocompatibility testing. The cells are found able to grow and attach in first day observation and dramatically increase after 3 days observation. This indicates that the film that is produced from wild silkworm cocoon of *Attacus atlas* has excellent biocompatibility.

1. Introduction

Diverse areas come together in an effort to maintain, improve, repair or replace biological function. Despite the remarkable of man-made synthetic biomaterial, their applications have been limited by challenges in biocompatibility, biodegradability and bioresorbability. Keys for improvement lies in the development of functional materials that can interact with biological systems [1].

Virgin silks, is having an issues of biocompatibility if uses as sutures, with reactions from delayed hypersensitivity to acute and chronic process of inflammatory. Degummed silk incorporated directly into biomaterial architectures. The absence of the silk sericin that is released by degummed silk fiber, demonstrate minimum reaction of inflammatory, which enables successful implantation and cell culture. silk for biomaterial requires the separation of sericin from fibroin [1].

The biomaterial extracted from silk can be produce in the form of particle, fiber, hydrogels, scaffold, and film [1, 2]. Some silks already explored for biomaterial such as *Bombyx mori* from domesticated mulberry silkworm or from non-mulberry silk such as *Antheraea mylitta*, *Antheraea assamenis*, *Antheraea pernyi*, *Philosamia ricini*, *Samia cynthia ricini* [1]. Other African source of non-mulberry wild silkworm cocoon also come in to attention like *Gonometa postica*, *Epiphora bauhini*, *Anaphe*



panda, and Argemamimosae [3]. In Japan, The wild silk from species Samiacyn-thiaricini was well studied to obtain the fibroin from its cocoo[4].

In Korea, the biomaterial film was developed by using fibroin from *Antheraea pernyi*. The cocoon was degummed in solution of calcium nitrate, whased with mixture of surfactant that nonionic and sodium bicarbonate [5]

Wild silk also become attention for the researchers in the USA. Cocoon from wild insects such as *H. euryalus*, *E. calleta*, *R. lebeau*, *A. oculae*, *H. gloveri* and *C. multifenestrata* were investigated and degummed protocol was treating with ethylenediamine and sodium carbonate solution for 50 min at 80°C with the cocoon to solution ratio of 1:20 . The degummed silk was thoroughly washed and dried [6].

It is a need in recent year to provide film in medicine for drug release surface engineering. Layer by layer thin film assemblies was introduced because possible to control composition in precision and can be incorporate with snall molecules, heparin, peptides, protein and enzymes without activity lost. Nano scale thin coatings of silk fibroin could be formed by stepwise deposition using an aqueous process. The fibroin protein adsorbed onto substrates spontaneously and the thickness could be controlled by dipping solution and coating dehydration method. The silk protein locks in the coatings due to physical crosslinks of β -sheet crystals resulting in stable material coatings that do not cross linking reactions [7].

In this research, silk was obtained from the Cocoon of *Atacus atlas* which is Indigenous from Indonesia. Biofilm will be produced from this cocoon for biocompatible biomaterial.

2. Experimental

Indonesian indigenous wild silkworm cocoon of *Attacus atlas* was prepared for this purpose (Fig. 1). The solution of NaOH with concentration in the range of 0 - 1 M were prepared for the degumming process in 1 hour and the best result from that range was used for the degumming protocol of the cocoon. The result of degumming process was observed in detail by using scanning electron microscope (SEM). The fiber obtained from degumming process was cleaning by using water at 70°C. The hot ceramic plate with temperature around 60°C was prepared and the mixture was dropped in series on the hot plate to obtain film as desired thickness. For the purpose of sterilization, then the film was put in 70% ethanol for 24 hours. After that the film was washed with solution of PBS. The film was introduce in cultivation media of COS-1 cell suspension for biocompatibility testing in the Chamber (humidity 95%, 5% CO₂, 37°C) for 1 day and 3 days testing. The COS-1 cells are cells of green monkey kidney (African). This cell with vector that require SV40 T antigen, suitable for transfection. The biocompatible characteristic (cell attachment and cell growth) were observed by using fluorescence microscope IX-71 (Olympus Japan). The sample should be transferred first in to PBS and washing it prior microscope observation.



Figure. 1 Indigenous Indonesian wild silkworm of *Attacus atlas* (a) and cocoon of wild silkworm *Attacus atlas* (b)

3. Result and Discussion

It is good to inform in this report that previously many degumming protocols are already establish for specific cocoon. The simplest method is by boiling [8]. Other method is by using Na_2CO_3 [9]. It was reported that degumming can also be performed by using urea that was developed because the level of degumming using boiling water is depending on the treatment time [10]. Degumming of the cocoon is performed traditionally by soap and can achieve optimum weight loss of at 30% [10]. There was a study that the removal of sericin by using an enzymes (protease and lipase). Degumming by using enzyme involves the proteolytic degradation of sericin [10]. All the above mentioned protocol for degumming have been applied for cocoon of *Attacus atlas* and was found not success to release the fiber from the cocoon.

The degumming process with NaOH treatment then is introduced in this research to overcome the difficulty of degumming cocoon of *Attacus atlas*. Good result in releasing fiber from the bound in the cocoon is found with concentration 0.1M NaOH as can be seen at Fig 2. If more higher concentration to apply such as at 1 M NaOH, would made the cocoon completely soluble in to the solution. But if the low concentration to apply such as 0.01 M or 0.001 M the solution will not effect on the releasing of the fiber from the cocoon as well as jus by using water (0 M NaOH). The detail result of degumming with 0.1 M NaOH can be observed in Fig.3. Fine fiber is found released from the bound of the cocoon.

The film has been soaked in alcohol for 1 day and appears to be stable as can be seen in the Fig. 4. Same sample with different thickness that was obtained from multiple dripping at temperature about 60 °C were also found very stable due to hydrophobic interactions. The films supported hMSC adhesion and proliferation [11]. The cells are found to attach and grow on the surface of the film that was made from silkworm cocoons of *Attacus atlas*. The cell are well grow at the the 1st day (Fig. 5 a) and continuously grow as can be proofed at the 3rd day in the Fig. 5b. This is an indication that the biofilm made from wild silkworm cocoon of *Attacus atlas* has good compatibility.

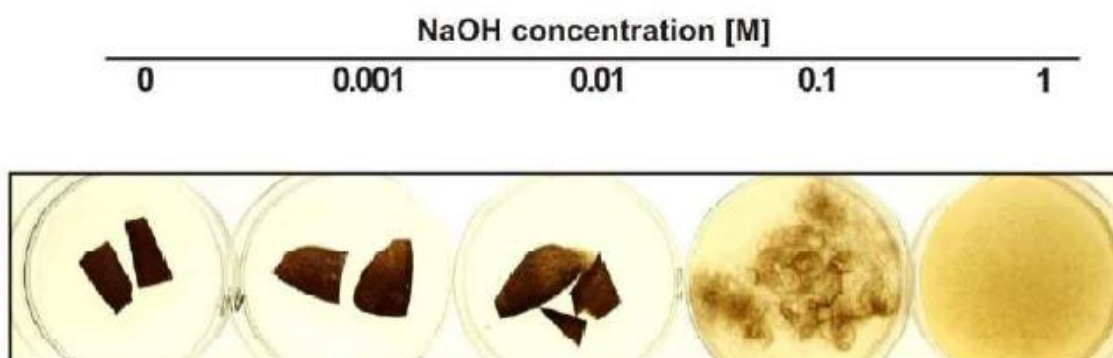


Figure 2 The Appearance of degumming process of cocoon of *Attacus atlas* at some variations concentration of NaOH.

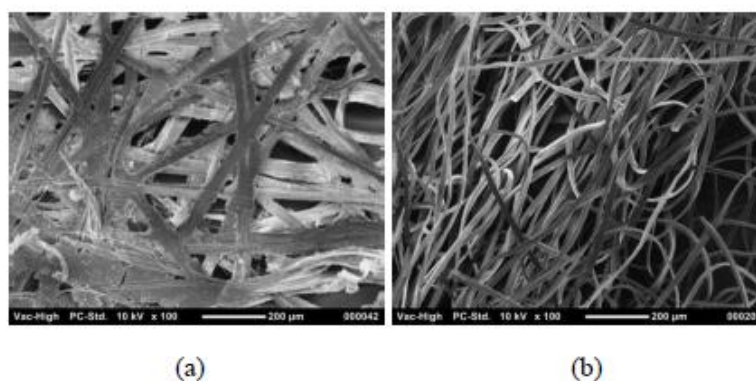


Figure 3 Texture fiber of cocoon *Attacus atlas* before degumming (a) and after degumming with 0.1M NaOH for 1 hour

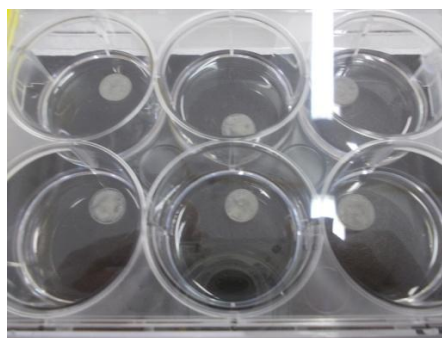


Figure 4 The film has been soaked in alcohol for 1 day and appears to be stable

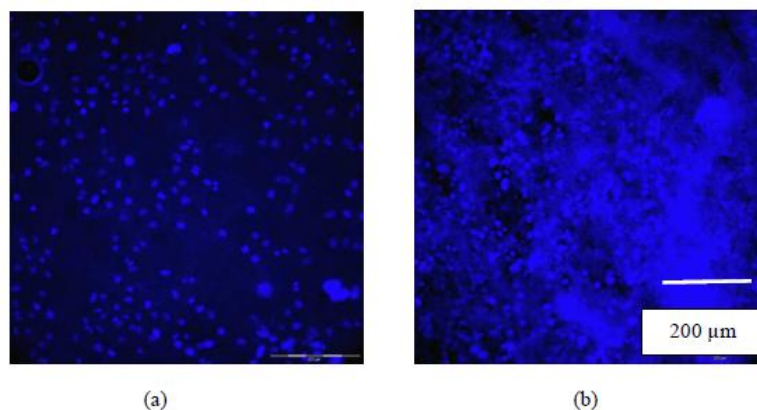


Figure 5 Cell are found well attached and grow on of the surface of the film in the 1st day (a). Remarkable cell growth in the 3rd day was observed on the surface of the film(b)

4. Conclusion

The Cocoon from wild silkworm cocoon of *Attacus atlas* can be processed for biocompatible film for medical application. The cocoon should be degummed by using 0.1M NaOH for 1 hour. The Film is produced by grinding the degummed fiber in wet condition and dripping in series on a hot ceramic plate at 60°C. The resulting film has proved biocompatible and stable

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