Biocompatible Film Made from Silkworm Cocoon

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Abstract—A biocompatible film is needed to cover an open or wide wounds. In this research, biocompatible film is created from cocoon of silkworm Bombyx mori. The sericin and fibroin proteins of the cocoon were separated. Only fibroin protein of the cocoon was used for this purpose. The fibroin was ground mechanically in water. The resulting fibroin solution was dropped onto a glass surface and heated at 60°C until solidify to become a film. The microstructure of the membrane was investigated by using optical microscope. The human osteosarcoma cell line (U2OS) was cultivated for 24 hours. It was found that the cells are able to attach and grow on the fibroin film of Bombyx mori during following 24 hours as documented by microscopic observation. This is an indication that the fibers may be used as degradable biomaterial.

Index Terms—cocoon, silk, fibroin, film, biocompatibility

I. INTRODUCTION

Some species of Lepidoptera caterpillars and also spider produces fibers called silk [1]. Silkworm Bombyx mori is a species that is well known and has undergone domestication. The silkworm will produce a cocoon as shown in Fig. 1. Besides already known for the production of textiles, fiber from the species of Bombyx mori silk already used for sutures [1]. The silk fibers then become attention for providing biomaterials for other purposes.

It has been proven that the silk fiber (from the species of Bombyx mori) is suitable as scaffolding material (3-D scaffolding) which allows for the cells to grow and spread along the fibers and after that grew to cover the entire surface and subsequently forming tissues [2].

Silk can also be used for implants in healing of critical size bone damage. This demonstrates that the feasibility

of silk-based implants for bone to form bone tissue that mechanically stable for corresponding period [3].

There are two major proteins in the silk of the cocoon namely fibroin and sericin. Sericin binds fibroin into one unified to form a cocoon for protection of the caterpillar inside the cocoon [1]. Sericin is a protein that acts like an adhesive (gum) and are antigenic. The sericin is enveloping the fibroin which is the core of the filament silk fibers. Fibroin is formed from the b-sheet crystals and part semi crystals that influence its elasticity [1].

Before further processing, the part of sericin of the fiber must be removed in order to obtain the fibroin (degummed). Generally the sericin in the cocoon is removed by boiling [4]. But in practice sericin will not be eliminated completely by just simply boiling.

Other methods to remove parts sericin has been found, for example by using Na₂CO₃ and then washed with water. Bombyx mori cocoons were boiled in an aqueous solution of Na₂CO₃, and rinsed with water to extract sericin and other contaminating proteins. Purified silk fibroin was solubilized in LiBr solution and dialyzed against water and 2-orpholinoethanesulfonic acid buffer, NaCl, and buffer. Silk fibroin solutions were dialyzed
against water and then lyophilized and redissolved in hexafluoro-2-propanol [3].

Other method for degumming is dried Bombyx mori silkworm cocoons were cut into small pieces and treated with boiling in aqueous solution of sodium carbonate with stirring. The mass was repeatedly washed with distilled water to remove the glue-like sericin protein and dried in hot air oven. Silk fibroin solution was prepared by dissolving of degummed silk in LiBr solution. The fibroin solution was dialyzed in a cellulose membrane based dialysis against deionized water in order to remove LiBr. After dialysis, silk fibroin solution was centrifuged [5].

A more simple degumming protocol can be prepared from Bombyx mori cocoons. cocoons were washed and dissolved in LiBr and dialyzed under osmotic pressure resulting in silk fibroin solutions [6].

For degumming method with Na$_2$CO$_3$ solution, cocoons were first boiled in water and then dried in an oven to obtain raw silk fibers. These fibers were degummed with Na$_2$CO$_3$ solution at 100°C and then rinsed with warm water. De-gummed silk was dissolved in a ternary solvent system of CaCl$_2$, ethanol, and H$_2$O. This solution was then dialyzed in water using a cellulose tubular membrane [7].

It was reported also that degumming can also be performed by using urea [8]. Sericins comprises of more random structures and possible to be hydrolyzed just by boiling in water. However, the level of degumming using boiling water is depending mainly on the treatment time [8].

Degumming method for silk is traditionally carried out by soap or soda ash method. In this degumming method, a weight loss of maximum 30% would normally obtained and reach a complete removal of sericin. Sericin is emulsified by the soap and removed from the silk fibre. The presence of alkalis and soap in the wastewater of degumming process raise a pollution issue [8].

There are various studies that have been dealt with the removal of sericin by using enzymes. For examples protease and lipase as degumming agents. Degumming by using enzyme involves the proteolytic degradation of sericin 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 is formed when substrate molecule fits into the active sites of the enzyme’s molecular structure. This complex then is broken and yields an end product and the original enzyme molecule is reproduced. Enzymes treatment can reduce energy consumption because operates under mild conditions and low temperatures. However, the enzyme degummed silk with lower performance, hight cost and difficult to handle which is limited the application of enzymes for degumming of silk [8].

For physical property enhancement, the acidic agent such as tartaric and citric acid was used for degumming and finishing the silk. The action of organic acids is less aggressive than an action by alkali solution. the high performance on degumming is achieved by tartaric acid in terms of sericin removal efficiency. The dry and wet resiliency of finished silk was remarkably increased with citric acid treatment. However, acid also causes the damage on the fibroin [8].

Alkaline processing is common chemical treatments which is aimed to increase the surface roughness of natural fibre for results in better mechanical interlocking. During degummed by an alkaline solution, a non-covalent bonds of silk fibroins are then modified and thus to cause the swell of the fibre. The swelling effect of the fibre is mainly governed by the difference of osmotic pressure arising between the fibre and the solution to form the protein salts. Alkalis such as NaOH (Sodium hydroxide) is commonly used now days for degumming. However, these strong alkali treatments impose a relatively harsh irritation to silk fibroins [8].

The purpose of this research is to produce films from silkworm cocoon of Bombyx mori that are biocompatible as a cover of wide wound where the process of suturing is not possible. The degumming process is developed from alkali treatment by using NaOH. This research is a complement and continuation of previous studies [9], [10] to produce various biomaterials from silkworm cocoon.

II. EXPERIMENTAL

Silkworm cocoons (obtained from sources in Indonesia) were immersed in a solution with a concentration of 0.01 M NaOH and boiling for 1 hour. Part of the cocoon that insoluble was washed with water (70°C), and then crushed mechanically in the water using glass mandrel (Fig. 2). The mixture was subsequently dripped over the glass plate with a temperature of around 60°C (Fig. 3).

The film obtained was sterilized with 70% ethanol for 1 day at room temperature. The film was washed with PBS solution then was introduced into COS-1 cell suspension in the cultivation media. Afterward the film is placed in a chamber with a setting of 5% CO$_2$, 37°C and humidity 95% for 2 hours.

![Figure 2. Glass mandrel for grinding the fibroin](image)

![Figure 3. Film formation by dripping a solution of fibroin on a glass plate with a temperature of about 60°C.](image)
The cultivation media was given a supplement of 10% FBS. Cultivation process was done for 1 day. For the observation of cells, the sample is taken out from cultivation media and transferred into PBS and cleaned them by washing process. Fluorescence microscope was used to observe cell growth (IX-71, Olympus Japan) as biocompatibility characteristics.

III. RESULT AND DISCUSSION

The degumming process that is introduced in this research is successful in eliminating the sericin from the silk fibre as can be seen in Fig. 4.

(a) Before degumming
(b) After degumming

Figure 4. The Bombyx mori silk fibre (a) before degumming. (b) After degumming

The biocompatible films are found stable after soaked in alcohol for 1 day. The result of the biocompatible film from this research is presented in Fig. 5. The film appears to be stable, although it has been soaked in alcohol for 1 day. It can also be noted here that the film remains stable if the thickness is increased by multiple dripping on a glass surface at temperature of about 60°C. The microstructure shows that the parts of the film are well bound therefore not easily torn apart (Fig. 6). It is found that the cells are able to attach and grow on the surface of the film made from silkworm cocoons of Bombyx mori. This is an indication that the film has good compatibility (Fig. 7).

IV. CONCLUSION

Biocompatible films can be made using silkworm cocoons of Bombyx mori. Film is made through the process of removing sericin by dissolving with NaOH solution (0.01 M). Fibroin solution is made by mechanically destroying in the mandrel glass continued by dripping on a glass surface with a temperature around 60°C. The resulting film has proved to be stable and biocompatible.

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