Morphology, Chemical Elements Composition, and Biocompatibility of Indonesian Wild Silkworm Cocoon

Tjokorda Gde Tirta Nindhia[1], Zdenek Knejzlik[2], Tomas Ruml[2]

1. Department of Mechanical Engineering, Engineering Faculty, Udayana University, Jimbaran, Bali, Indonesia.
Fax: +62-0361- 8953472
E-mail: nindhia@yahoo.com

2. Faculty of Food and Biochemical Technology, Technicka 5, 166 28 Prague,
Czech Republic

Abstrak

In this article, the morphology of two indigenous Indonesian wild silkworm cocoons from the species of Cricula trifenestrata and Attacus atlas were identified and compared with common silkworm cocoon of Bombyx mori that was also grown in Indonesia. The equipment that was utilized for identification processes was scanning electron microscope (SEM). The Laser Induced Breakdown Spectroscopy (LIBs) was used to identify the elements content in the respective silkworm cocoon. The Cell from the Type of U-2 OS was used for test the biocompatibility of the silkworm cocoon under cell culturing. The morphologies are presented in the report and the chemical element compositions are reported respectively. It is Interesting to be explored that both Indonesian wild silkworm cocoon have good biocompatibility as the domesticated silkworm Bombyx mori.

Keywords: Morphology, wild, silkworm, cocoon, Indonesia, biocompatibility

Introduction

Silks are superior material in mechanically, biocompatible and degradable there fore offer a wide range of properties (Veparia & Kaplan, 2007). The mechanical properties of silks in fiber in terms of energy absorbed before failure exceed all other natural polymers and synthetic materials, even high-performance fibers of Kevlar (Meinela *et al.*, 2005).

As reported in many publications, the silk from the species of Bombyx mori is well known for its biocompatibility as biomaterial. The domesticated Bombyx mori silk has been used commercially as sutures in biomedical (Altman *et al*., 2003).

Silk then have more attention as biomaterial since many researcher recently was demonstrated that silk is suitable for 3-D scaffolding material. As a scaffold, the cell growth and spread along the fiber, and the next is covered all the surface of the fiber and then spread to fill the gap to form structure of the tissue (Unger[1] *et al.*, 2004). It was also reported that the human cell was adhere, grow and spread on three dimensional silk fibroin produced from cocoons of Bombyx mori(Unger[2] *et a.l*, 2004).

The researches for utilization of silkworm cocoon as biomaterial as above mentioned until know just put attention on one species of silkworm cocoon that is form the species of Bombyx mori. Other type of silkworm cocoon especially from the wild that is not domesticated yet should be explored in other to find more suitable silk material for the better or even more superior biocompatibility. It is the purpose of this research to observe the Indonesian source of wild silkworm cocoons from the species of Cricula trifenestrata and Attacus atlas to be investigated its morphology, chemical elements composition and biocompatibility.

Experimental

The wild Silkworm cocoon form the species of Cricula trifenestrata (Fig. 1a) and Attacus atlas (Fig. 1b) were obtained from Indonesian sources. The domesticated silkworm cocoon of Bombyx mori that was also form Indonesian source (Fig.1c) was also included in this research for the comparison as a standard material.

The observation on morphology was conducted by using scanning electron microscope SEM). The photo was taken and the diameter was measured. It was selected 100 single fibers from the cocoon randomly

and the result was presented in histogram for easy comparison.

(a)

(b)

(c)

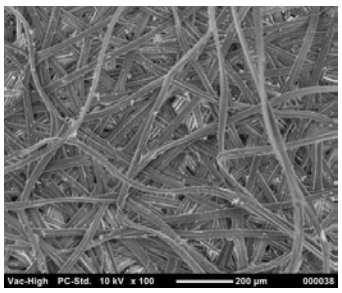
Fig. 1. (a) Cocoon of Cricula trifenestrata, (b) Attacus atlas, and (c) Bombyx mori

The laser induced breakdown spectroscopy (LIBs) was used to identify the element content in the respective silkworm cocoon. Due to limitation of the space for this article, only the element from the wave length range from 0 to 500 nm was presented.

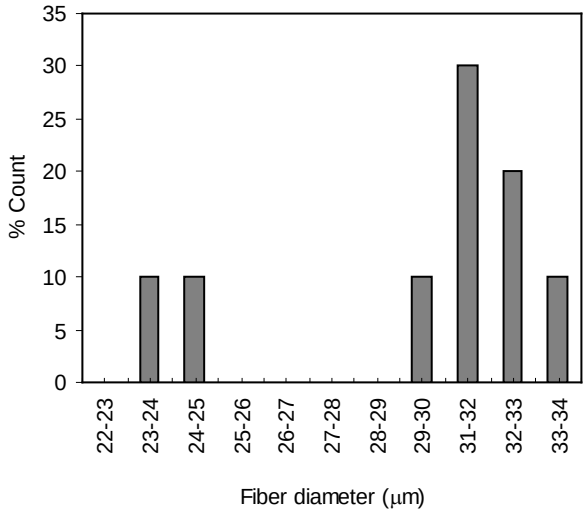
For the purpose of biocompatibility test, the whole silkworm cocoon should undergo degumming process. The detail of the degumming process will be explain in separate article but related in this article. The degummed silk was put in to the liquid of cell culture (DMEM Q media^{tm}) from SIGMA. The cell that was used is U-2 OS from ATCC (USA) and let to grow for 2 days. Observation of the cell growth was conducted by using fluorescence microscope with prior fixed and staining the cell.

Result and discussion

The morphology of the fiber from the Bombyx mori is presented in Fig.2a. It is found by measurement that the fiber diameter is 30% appear in frequency of the histogram for the size 31-32 μm (Fig 2). This is mean that the fiber size is smaller that both Cricula trifenestrata and Attacus atlas. The Cricula trifenestrata is found with diameter 55-56μm or 58-59μm (appears the same 20% in frequency of the histogram) as depicted in Fig.3a and Fig 3b. The diameter of the Attacus attlas is seem in between of Bombyx mori and Cricula trifenestrata that is 40-41 μm (appear 30% in frequency of the histogram in Fig. 4)

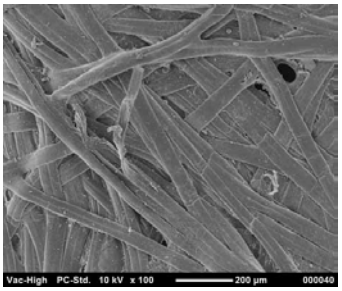


(a)



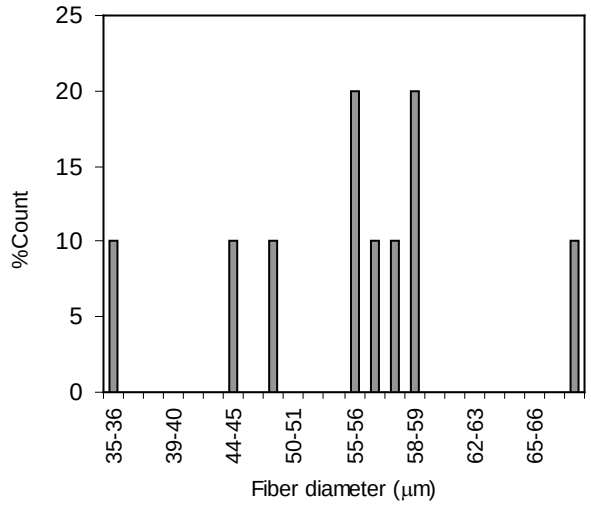
(b)

Fig. 2. (a) Marphology of Bombyx mori (b) Histogram of the diameter of the fiber of Bombyx mori



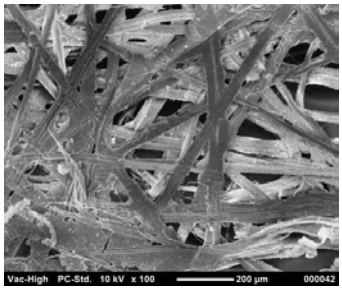
(a)

Fig. 3. (a) Marphology of Cricula trifenestrata

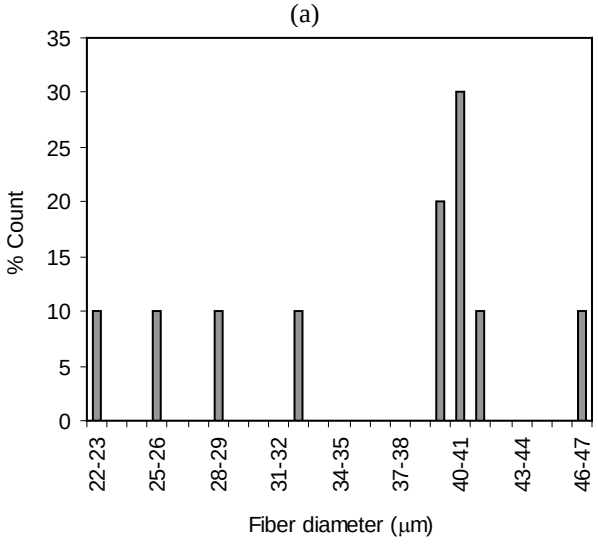


(b)

Fig. 3. (b) Histogram of the diameter of the fiber of Cricula trifenestrata



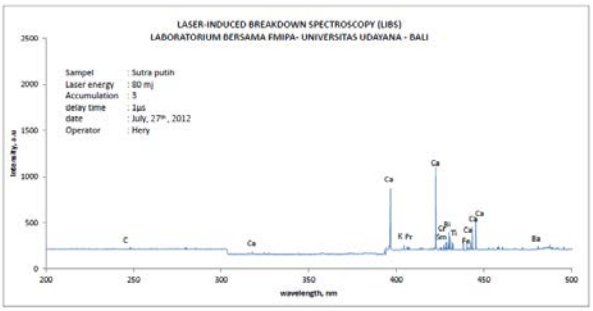
(a)



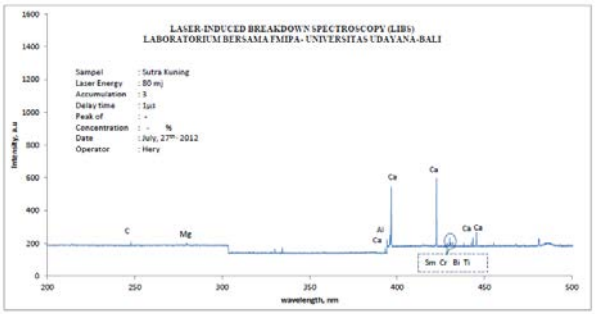
(b)

Fig. 4. (a) Marphology of Attacus atlas
(b) Histogram of the diameter of the fiber of Attacus atlas

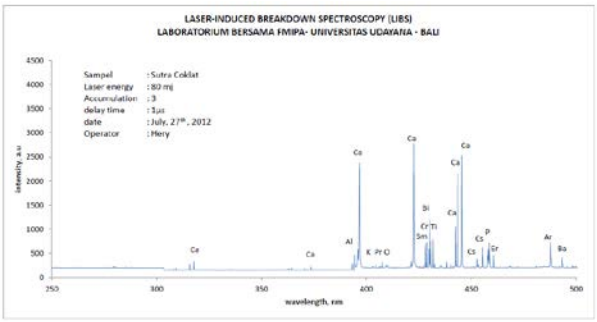
It can be proofed by The laser induced breakdown spectroscopy (LIBs) that the chemical element content each cocoon are found different as depicted in Fig 5. The Chemical element content in the Bombyx mori are: C, Ca, K, Pr, Sm, Cr, Bi, Ti, Fe, Ba. Chemical elements content for the Cricula trifenestrata are: C, Mg, Al, Sm, Cr, Bi, Ti. There are about 14 elements that are found in the Attacus atlas namely: Ca, Al, K, Pr, O, Sm, Cr, Bi, Ti, Cs, P, Er, Af, Ba



(a)



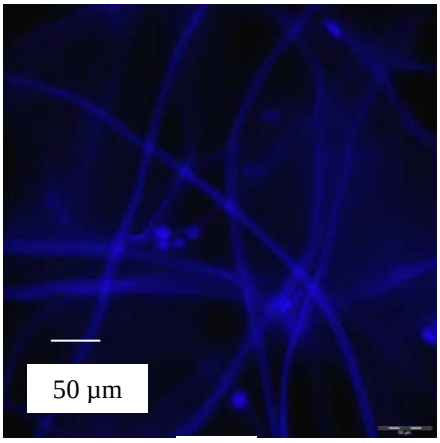
(b)



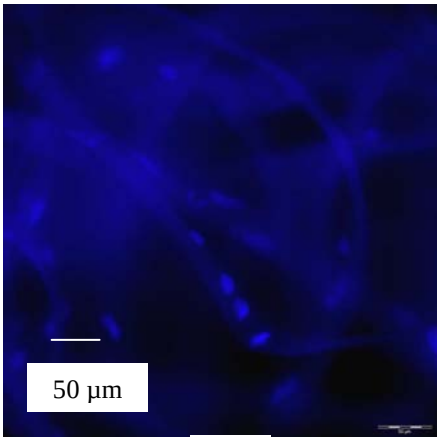
(c)

Fig. 5. Cemical analyses result by using Laser induced breakdown spectroscopy (LIBs) (a) Bombyx mori (b) Cricula trifenestrata (c) Attacus atlas

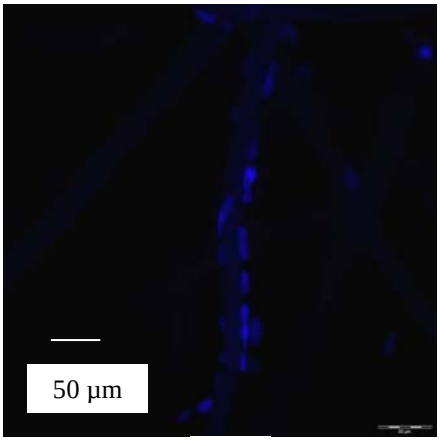
It is interesting to discuses here related the biocompatibility of the wild silkworm cocoons of Indonesia. Fig. 6 is the microscope observation of the growth of the cell in the respective silkworm cocoon for 2 days. It is clearly observed that the cell are found more in the both of Cricula trifenestrata and Attacus atlas (Fig. 6b and 6c) comparing in the Bombyx mori. This condition can be directed to the result that wild silkworm cocoon from Indonesia are more biocompatible comparing the Bombyx mori that usually use in recent day for biomaterial research.



(a)



(b)



(c)

Fig. 6. Growth of the cells (a) Bombyx mori (b) Cricula trifenestrata (c) Attacus atlas

Conclusion

The morphology of the Silkworm cocoon of Cricula trifenestrata and Attacus atlas were found different with Bombyx mori. The diameter of Bombyx mori fiber is about half of the Cricula trifenestrata or Attacus atlas. The chemical element composition are proofed different but all are found having good biocompatibility for cell culturing with note that it seem both Indonesia wild silkworm cocoon having better biocompatibility than Bombyx mori.

Acknowledgements

The authors acknowledge financial support received from Directorate General of Higher Education of The Ministry Education and Culture The Republic of Indonesia trough Scheme of Academic Mobility and Exchange program under contract number: 2033.26/E4.2/2012 dated 2nd July 2012. Partial support from the Institute of Chemical Technology of Prague also very much acknowledged.
Special thank addressed to Prof. Ing. Katerina Demnerova, CSc. Together with Ing. Jan Lipov, Ph.D For the permisison to use all research facility in the Departement of Biochemical and Microbiology Institute techmology of Prague, Czeck Republic.

References

Altman, G.H., Diaz, F.,Jakuba, C.,Calabro, T., Horan, R. L., Chen, J., Lu, H., Richmond, J. , Kaplan, D. L. Silk-based biomaterials, Biomaterials, 24, 401–416, (2003)

Meinela, L., Fajardod, r., Hofmanna, S., Langerc, R., Chene, J., Snyderd, B., Novakovic, G.B., Kaplan, D., Silk implants for the healing of critical size bone defects, Bone, 37, 688–698, (2005)

Unger[1] , R.E., Petersa, K., Wolf, M., Motta, A., Migliaresi, C., Kirkpatrick, C.J., Endothelialization of a non-woven silk fibroin net for use in tissue engineering: growth and gene regulation of human endothelial cells, Biomaterials 25 , 5137–5146, (2004)

Unger[2], R.E., Wolf, M., Peters, K., Motta, A.,Migliaresi, C., Kirkpatrick, C.J., Growth of human cells on a non-woven silk fibroin net: a potential for use in tissue engineering, Biomaterials, 25, 1069–1075, (2004)

Veparia, C., Kaplan, D.L., Silk as a biomaterial, Prog. Polym. Sci., 32 . 991–1007, (2007)