# BIODEGRADATION OF A SILKWORM SILK/PLA COMPOSITE

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## Abstract

Animal silks and Poly(lactic acid)(PLA) are potential materials for biomedical and bioengineering applications. Biodegradability of these materials thus is important to minimize any extra pain of patients with bone defects due to second operations for removing those non-biodegradable implants. However, the control of their biodegradability and mechanical properties is essential to ensure a smooth load transfer from a depredating implant to neo-tissue. In this study, a biodegradation test on silk/PLA biocomposites was performed. Physical and mechanical properties, pH condition of the surrounding fluid and the morphology of fractured samples were studied at specific time points. It was found that there were no significant differences

between the pH values of the solution and weight loss for both pure PLA samples and silk/PLA biocomposites. Moreover, with the reinforcement of silk fiber, stiffness and ductility of PLA were enhanced and a faster biodegradation rate was observed within the 4-month biodegradation period. It can be concluded that the biodegradation rate of implants can be altered and their mechanical properties can be enhanced by incorporation of silk fiber. This is a potential solution to match with the degradation rate of PLA to the regeneration rate of neo-tissues.

**Keywords:** A. Polymer-matrix composites (PMCs); B. Mechanical properties; B. Physical properties; D. Surface analysis.

## 1. Introduction

Traditional non-corrosive metallic materials such as Titanium, Nickel-titanium, stainless steel and etc. are desirable choices for many load-bearing biomedical prostheses applications. Since body fluid contains various types of ions, metal implants are susceptible to ion attack and may release potential toxic or harmful by-products such as ions, chemical compounds and particulate debris. If these materials are inappropriately used inside the human body, adverse biological responses will occur. Certain polymers have been designed to undergo controllable degradation. Among these polymers, poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers have been the most widely used to date. They are found to be biocompatible while at the same time being able to provide suitable mechanical strength for the implant applications. During degradation, these materials degrade into smaller fragments as well as monomers, such as lactic acid, that can be eliminated by normal metabolic processes of the body.

Nowadays, many biodegradable polymers are used for sutures, controlled drug delivery, tissue engineering and fracture fixation. In recent years, different types of biodegradable polymers have been studied extensively for scaffold fabrication and musculoskeletal tissue engineering. Apart from a wide range of mechanical properties, biocompatibility and biodegradability play important roles in the engineering process of the regeneration of neo-tissues. These properties can subsequently affect cells vitality, cell growth and even host response. It is believed that the ideal in vivo degradation rate may be similar or slightly less than the rate of tissue formation. When large part of load-bearing tissues, such as bone, tendons or ligaments etc. are removed due to serious fragmentation or infection, a substitute biomaterial is often required to aid the restoration of tissue function. Slowly degrading biomaterials, which can maintain tissue integrity of implantation site and continually transferring the load-bearing burden to the regenerating biological functional tissues are highly desired. This gradual load transfer at a predictable and controllable rate is especially important to mechanical sensitive tissues. In addition, the mechanical integrity of implants is important to withstand various types of stresses, guide the direction of tissue generation and align mechanical axis at the implantation site.  All of them are prerequisite requirements for biomaterials. Therefore, the control of biodegradation rate and mechanical properties is essential to maintain the structural integrity until the loaded cells adapt to the environment and excrete sufficient amount of extracellular matrix etc. and eventually the implants are replaced by newly formed tissues.

As aforementioned, PLA is a widely used biodegradable polymer for suture and scaffold fabrication. It possesses good mechanical properties and biocompatibility. However, when PLA is fabricated as porous scaffold, its mechanical properties will

drop dramatically and may not provide sufficient strength for load-bearing applications. To address this problem, silk fiber may be a good alternative as reinforcement to enhance the strength of PLA. Silk fiber has long been used as surgical sutures in medical history; their substantial biomechanical properties are the main advantage for utilizing them in biomedical applications [1-7]. According to previous literatures, silk fiber is a promising reinforcement for different types of composites due to its unique structure and significant similarity of physical and mechanical properties as a natural fiber [8-11]. For these reasons, biodegradability and mechanical property tests of silk/PLA biocomposites were then examined under different types of mechanical and biodegradation test.

## 2. Experimental Procedures

### 2.1 Preparation of samples

Natural tussah silkworm silk fiber (hereafter called “silk fiber”) was used as reinforcement for fabricating a completely biodegradable silk/PLA biocomposite. Silk fiber was cut gently into short fiber fractions in the length of 5 mm to make sure that the fiber was not stressed plastically during the fabrication process. A thermoplastic, PLA, was used as matrix in this study. Before fabricating the biocomposite, silk fiber and PLA pellets first underwent a dry treatment with heat applied (80 $^{o}$C) for 24 hours in an oven so as to remove excessive moisture. This step should be emphasized, otherwise the properties of PLA would be strongly affected due to the formation of voids after curing. All sets of samples were fabricated by extrusion and injection molding techniques to ensure that consistent results could be obtained. The silk fiber and PLA matrix were mixed at the ratio of 5 wt. % to 95 wt. % and fed into a Hakke MiniLab twin-screw micro extruder. A uniform temperature of 180 $^{o}$C was maintained at all zones inside the

machine, and the screw speed and the mixing duration were set to be 100 rpm and 10 minutes, respectively. The first run of the extrusion was discarded and the strands of the extrusion products were then directly collected by a pre-heated injection cylinder for further injection molding. The molten mixture was then transferred to a Thermo Hakke small scale injection-molding machine; the injection cylinder and the mold were pre-heated to desired temperatures of 200 $^{o}$C and 45 $^{o}$C respectively. The resultant biocomposite samples were in a dumbbell shape according to ASTM D638. The mechanical properties of 5 mm 5 wt% silk/PLA biocomposites (hereafter called "silk/PLA biocomposites") and pure PLA samples were then compared.

**2.2 Biodegradation test**

In this study, a total of 40 samples were fabricated: half of them were pure PLA samples and the others were silk/PLA biocomposites. In vitro biodegradation test was performed by making use of Dulbecco's Phosphate Buffered Saline (PBS), which is balanced salt from Sigma in white dry powder form, of pH 7.4. In order to form 1xPBS solution, 9.6 grams of PBS powder was diluted with 1 L of de-ionized water. Two different types of dumbbell-shaped samples were stored in two separate 500 ml containers with 300 ml 1xPBS solution and the containers were stored in a humidified, thermo-stable and orbital-shaking incubator at 37 $^{o}$C for up to 16 weeks (around four months). During the first week of the test, pH values of 1xPBS solution in each of the containers were measured daily in order to understand the stability of the acidity of the solution. The used 1xPBS solution was replaced by fresh 1xPBS solution at the end of each week to mimic the fairly constant acidity environment in vivo. Four specific degradation time points were set at a 4-week interval, i.e. 4, 8, 12 and 16 weeks. At each time point, five

pure PLA samples and five silk/PLA biocomposites were taken out from the containers for physical and mechanical properties characterization.

### 2.3 Measurements

#### Physical properties characterization

The pH values of two different set of samples were checked weekly by pH meter and compared with the fresh 1xPBS solution as reference before being replaced by new 1xPBS solution. In addition, weight loss and changes in appearance of the samples due to the release of acidic products to the solution were examined at the specific testing time point (i.e. every four weeks).

#### Mechanical properties characterization

Tensile test was then carried out in order to examine the mechanical properties of pure PLA samples and silk/PLA biocomposites according to the ASTM standard by using the 50 kN MTS Alliance RT-50 tensile machine and extensometer. In order to maintain the accuracy of measurement, all testing samples were molded into a dumbbell shape and undergone the same ambient testing conditions of 22 $^{o}$C and 45 % humidity. The span length of the extensometer was 25 mm, and crosshead speed with a loading rate of 2 mm/min was used. A total of ten samples were tested at each specific time point, of which five were the pure PLA samples and the others were the silk/PLA biocomposites.

#### Fracture surface characterization

After the mechanical property tests, microscopic analysis was conducted with the help of Leica Stereoscan 440 scanning electron microscope (SEM) on the fracture surface of the samples to examine the failure surface structure and failure behavior induced by

previous tensile test. Analysis was performed at room temperature with tungsten filament, and an accelerating voltage of 20 kV was used to capture SEM images for both of the pure PLA sample and silk/PLA biocomposite. All specimens were viewed from the top of the fractured surface.

## 3. Results and Discussion

### 3.1 pH values, appearance and weight after biodegradation

The 1xPBS solution of pH 7.4 was used for in vitro biodegradation test at 37 $^{o}$C in a shaking incubator for 16 weeks. The used 1xPBS solution was replaced by fresh 1xPBS solution and pH value of the solution was checked weekly by a pH meter to study the effect of acidic degradation products. After the 16-week biodegradation test, the pH value of the 1xPBS solution remained almost same in the range of pH 7.37 and 7.41 for both pure PLA samples and silk/PLA biocomposites as shown in Table 1. Thus, it can be claimed that the degradation does not affect the pH value significantly. On the other hand, the appearances of pure PLA samples and silk/PLA biocomposites remained unchanged in the first 4 weeks of the test, and they started to change from transparent to translucent and from yellow to pale yellow in color respectively afterward. Moreover, the weight of two different types of samples remain unchanged at 1.3 g for the first 12 weeks and dropped slightly to 1.24 g for the pure PLA samples and 1.26 g for the silk/PLA biocomposites at the last time point (16 weeks) of the biodegradation test as listed in Table 2. Consequently, mass change with time was slow in general.

### 3.2 Tensile test

Every Implant is assumed to maintain their mechanical integrity at implantation site before its desired function has been fulfilled. Therefore, the mechanical properties of

pure PLA samples and silk/PLA biocomposites were examined at specific time point during the biodegradation test for indication of biodegradability and strength of the materials. Curves of ultimate tensile strength and modulus of elasticity against biodegradation time are as shown in Figures 1 and 2. Dotted line in the figures represents the mechanical property results of the silk/PLA biocomposites. For the ultimate tensile strength, the pure PLA samples degraded constantly against time whereas the silk/PLA biocomposites degraded in a faster rate in the first 2 weeks, followed by a constant rate of biodegradation. On the other hand, regarding the modulus of elasticity of the two different types of samples, it can be observed from Figure 2 that silk fiber enhanced the stiffness of PLA in the beginning, and the silk/PLA biocomposites degraded constantly with a relatively faster biodegradation rate than that of the pure PLA samples during the biodegradation test. Previous research work that investigated the degradation kinetics of biodegradable scaffolds has revealed that the method of decreasing the porosity of scaffolds to attain higher initial stiffness would result in faster degradation and shorten the life of scaffolds. Some proteinous materials such as silk fibroin, elastin and amino acids etc. were found to stimulate the production of enzymes from PLA-degrading micro-organisms [12-13]. Additionally, silk fiber is hydrophilic whereas PLA is hydrophobic polymer which can only absorb about 1 % water content; therefore, silk fiber will be the major factor affecting the water absorption ability of the composite. Increment on silk fiber content leads to a faster water absorption rate of the material due to its strong hydrophilic property, while for PLA, moisture susceptibility is the primary driving force towards biodegradation. Thereby, the area exposed to hydrolysis increased with the reinforcement of silk fiber, resulting in a higher biodegradation rate of the biocomposite. It can be claimed that the biodegradation rate of PLA could be improved and controlled by reinforcements of silk

fiber which induces porous structure to pure PLA material. By controlling the biodegradation rate of implant materials, the regeneration rate of neo-tissues at implantation site can be matched; load-bearing functions can be transferred desirably from the implants to the neo-tissues. The biodegradation mechanism of the two different types of samples can also be explained by SEM micrographs.

### 3.3 Fracture surface characterization

After tensile test, the morphology of the fracture surface of pure PLA samples and silk/PLA biocomposites was then sent for SEM investigations. Micrographs of two different types of samples after the biodegradation test at specific time points were as shown in Figures 3 to 7. As seen from Figure 3, the topology of fractured pure PLA samples and silk/PLA biocomposites before biodegradation test are relatively rough and no voids are observed when compared to those fractured samples during biodegradation test. According to previous literatures, the manufacturing process of the samples was in good condition, so, moisture absorption during fabrication did not occur in the samples. Apart from this, fiber network was able to reinforce the biocomposite during loading condition. The bridging effect was created to stop any crack propagation, which is well matched to the result obtained from the tensile test. Fiber fracture is mainly observed with few parts of fiber pull-out during the examination, which reflects that a good interfacial bonding between the fiber and matrix exist in the silk fiber/PLA biocomposite by using extrusion and injection molding techniques. Yet, better interfacial bonding between reinforcement and matrix can be further improved by applying some bio-based coupling agents or performing surface modification of the fiber before or during fabrication of the biocomposite [14-15].

Figures 4 to 7 represent the fractured pure PLA samples and silk/PLA biocomposites at specific biodegradation time points (4, 8, 12 and 16 weeks) respectively. It can be observed that the surface morphology of the pure PLA samples became smoother from 4 weeks up to 12 weeks, and it is obvious that from the mechanical property test, the ductility of the samples is increasing from the beginning of the test up to 12 weeks. Samples became relatively brittle at 16th week of the test. On the other hand, the silk/PLA biocomposites became more rubbery on the surface up to 8 weeks of the biodegradation test, which is mainly due to the serious water absorption problem. Besides, larger portion of PLA in the silk/PLA biocomposite was degraded at 12 and 16 weeks of the test as the interfacial bonding between silk fiber and PLA became weaker, and more water was absorbed by the fiber than before.

## 4. Conclusion

Pure PLA samples and silk/PLA biocomposites were examined under the in vitro biodegradation test for 16 weeks, and the physical and mechanical characterizations were performed at different specific time points. The pH value of the 1xPBS solution was fairly constant, which showed that the acidity of degradation product from both pure PLA samples and silk/PLA biocomposites are mild and do not affect the in vivo environment. The appearance of pure PLA samples and silk/PLA biocomposites started to change from transparent to translucent and from yellow to pale yellow in color respectively after the 4th week of the test. Moreover, mass change with time for both set of samples was slow in general. For the mechanical property test of the biodegraded pure PLA samples and silk/PLA biocomposites, stiffness and ductility of PLA were enhanced with the reinforcement of silk fiber. Biodegradation rate of the PLA was seen to be improved and controlled with the reinforcements of silk fiber which induces

porous structure to the matrix for faster rate of water intake due to its strong hydrophilic ability. It is concluded that silk fiber can be a potential candidate as reinforcement for biocomposite as the biodegradation rate of implants can be controlled so as to match with the regeneration duration of neo-tissues at implantation site to fulfill its desired function. Last but not the least, extrusion and injection molding techniques for fabricating biocomposites can enhance fiber dispersion, interfacial bonding between reinforcement and matrix, while voids and water absorption during fabrication can be largely avoided. Further improvement for the interfacial bonding of the biocomposite can be done by applying some bio-based coupling agents or surface modification of the fiber before or during fabrication of the biocomposite in order to produce high performance biocomposite.

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**References**


1. Meinel L., Hofmann S., Karageorgiou V., Kirker-Head C., McCool J., Gronowicz G., Zichner L., Langer R., Vunjak-Novakovic G. and Kaplan D.L.. The inflammatory responses to silk films in vitro and in vivo. Biomaterials 2005; 26: 147-155.
2. Dee K.C., Puleo D.A. and Bizios R.. An Introduction to Tissue-Biomaterial Interactions. United States of America: John Wiley & Sons, Inc.; 2002.
3. Wu L. and Ding J.. In vitro degradation of three-dimensional porous poly(D,L-lactide-co-glycolide) scaffolds for tissue engineering. Biomaterials 2004; 25: 5821-5830.
4. Black J.. Biological performance of materials: fundamentals of biocompatibility. 3 rd Edition. United States of America: Marcel Dekker, Inc.; 1999. Part II.: p. 27-128.
5. Biomaterials degradation: fundamental aspects and related clinical phenomena. In: Barbosa M.A., principal editor. The Netherlands: Elsevier Science Publishers B.V.; 1991.
6. Lu L., Peter S.J., Lyman M.D., Lai H.L., Leite S.M., Tamada J.A., Vacanti J.P., Langer R. and Mikos A.G.. In vitro degradation of porous poly(L-lactic acid) foams. Biomaterials 2000; 21: 1595-1605.
7. Lu L., Peter S.J., Lyman M.D., Lai H.L., Leite S.M., Tamada J.A., Uyama S., Vacanti J.P., Langer R. and Mikos A.G.. In vitro and in vivo degradation of porous poly(DL-lactic-co-glycolic acid) foams. Biomaterials 2000; 21: 1837-1845.
8. Priya S.P., Ramakrishna H.V. and Rai S.K.. Tensile, flexural, and chemical resistance properties of waste silk fabric-reinforced epoxy laminates. Journal of

Reinforced Plastics and Composites. Vol 24, pp 643-648, 2005.

9. Lee S.M., Cho D.H., Park W.H., Lee S.G., Han S.O. and Drzal L.T.. Novel silk/poly(butylenes succinate) biocomposites: the effect of short fiber content on their mechanical and thermal properties. Composites Science and Technology. Vol 65, pp 647-657, 2005.
10. Han S.O., Lee S.M., Park W.H. and Cho H.. Mechanical and thermal properties of waste silk fiber-reinforced poly(butylenes succinate) biocomposites. Journal of Applied Polymer Science. Vol 100, pp 4972-4980, 2006.
11. Lee K.G., Kweon H.Y., Yeo J.H., Woo S.O., Lee J.H. and Park Y.H.. Structural and physical properties of silk fibroin/alginate blend sponges. Journal of Applied Polymer Science. Vol 93, pp 2174-2179, 2004.
12. Agrawal C.M., McKinney J.S., Lanctot D. and Athanasiou K.A.. Effects of fluid flow on the in vitro degradation kinetics of biodegradable scaffolds for tissue engineering. Biomaterials 2000; 21: 2443-2452.
13. Tokiwa Y. and Calabia B.P.. Biodegradability and biodegradation of poly(lactide). Applied Microbiology and Biotechnology 2006; 72: 244-251.
14. Lee S.H. and Wang S.. Biodegradable polymers/ bamboo fiber biocomposite with bio-based coupling agent. Composites Part A: Applied Science and Manufacturing 2006; 37: 80-91.
15. Teramoto N., Urata K, Ozawa K. and Shibata M.. Biodegradation of aliphatic polyester composites reinforced by abaca fiber. Polymer Degradation and Stability 2004; 86: 401-409.

| Duration of test / Samples | Before test | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days |
|---|---|---|---|---|---|---|---|
| Pure PLA | 7.40 | 7.39 | 7.40 | 7.38 | 7.38 | 7.39 | 7.41 |
| Silk/PLA | 7.40 | 7.39 | 7.39 | 7.37 | 7.38 | 7.40 | 7.41 |

| Duration of test / Samples | 1 week | 2 weeks | 3 weeks | 4 weeks | 5 weeks | 6 weeks | 7 weeks | 8 weeks |
|---|---|---|---|---|---|---|---|---|
| Pure PLA | 7.39 | 7.38 | 7.37 | 7.40 | 7.39 | 7.37 | 7.38 | 7.37 |
| Silk/PLA | 7.37 | 7.38 | 7.38 | 7.41 | 7.41 | 7.37 | 7.37 | 7.37 |

| Duration of test / Samples | 9 weeks | 10 weeks | 11 weeks | 12 weeks | 13 weeks | 14 weeks | 15 weeks | 16 weeks |
|---|---|---|---|---|---|---|---|---|
| Pure PLA | 7.37 | 7.37 | 7.41 | 7.41 | 7.37 | 7.41 | 7.38 | 7.40 |
| Silk/PLA | 7.37 | 7.39 | 7.41 | 7.39 | 7.37 | 7.4 | 7.39 | 7.39 |

Table 1 pH values of 1xPBS solution during biodegradation test.

| Duration of test / Samples | Before test | 4 weeks | 8 weeks | 12 weeks | 16 weeks |
|---|---|---|---|---|---|
| Pure PLA | 1.30 g | 1.30 g | 1.30 g | 1.30 g | 1.24 g |
| Silk/PLA | 1.30 g | 1.30 g | 1.30 g | 1.30 g | 1.26 g |

Table 2 The weight of pure PLA samples and silk/PLA biocomposites during biodegradation test.

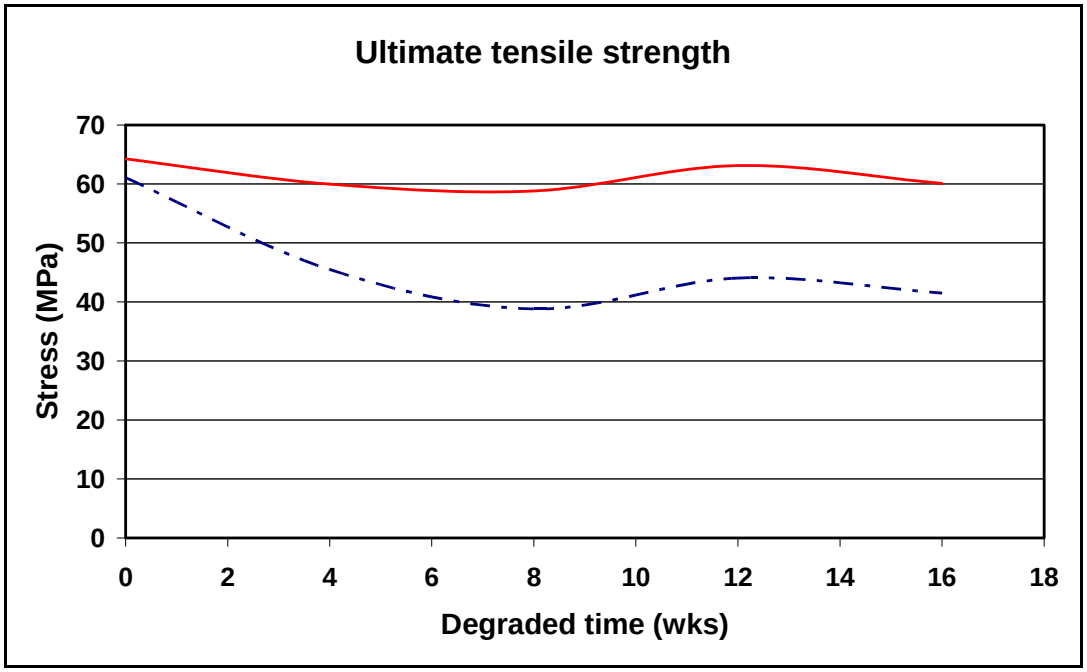


Fig. 1 Curves of ultimate tensile strength against biodegradation time of pure PLA samples and silk/PLA biocomposites (dotted line).

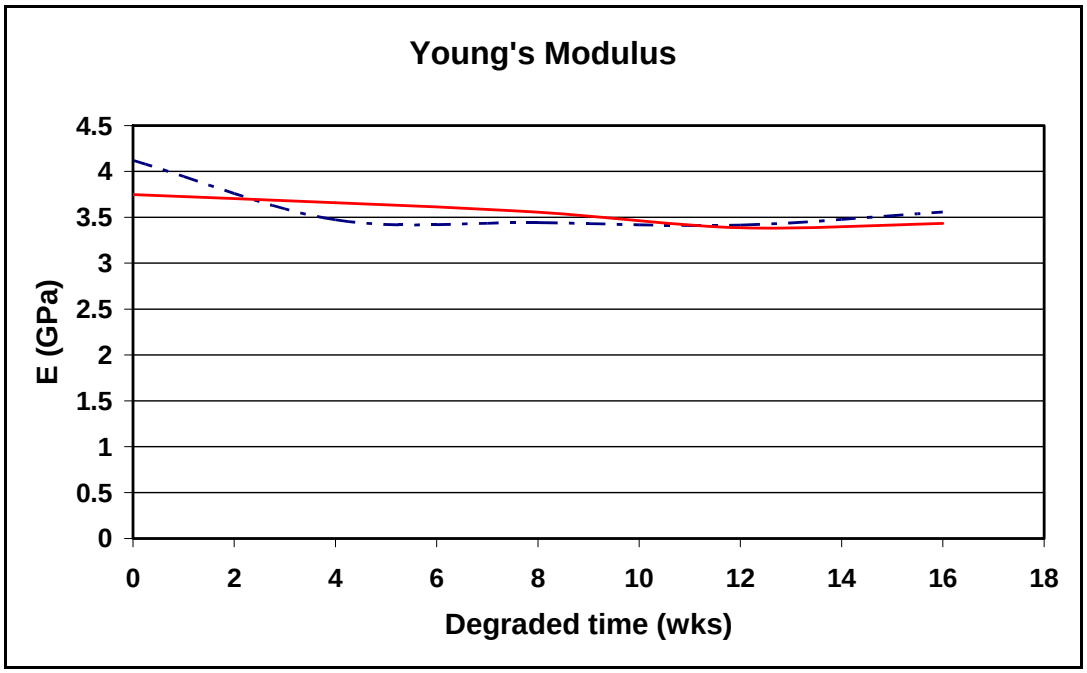


Fig. 2 Curves of modulus of elasticity against biodegradation time of pure PLA samples and silk/PLA biocomposites (dotted line).

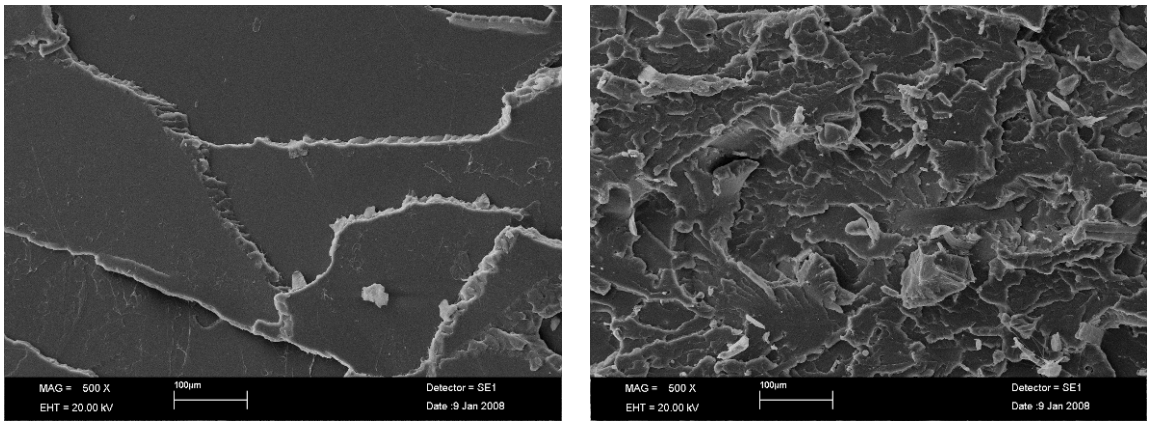


Fig. 3 SEM images of pure PLA samples (left) and silk/PLA biocomposites (right) before biodegradation test.

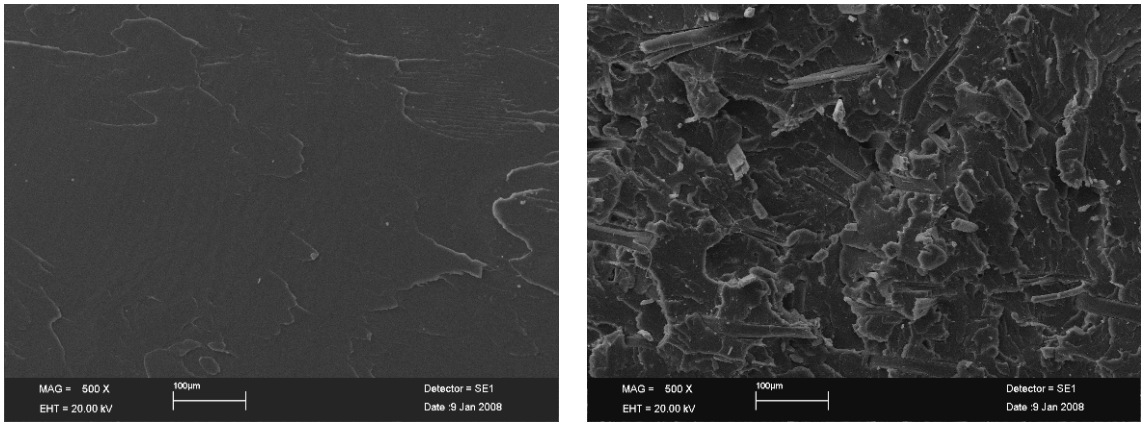


Fig. 4 SEM images of pure PLA samples (left) and silk/PLA biocomposites (right) after 4 weeks of biodegradation test.

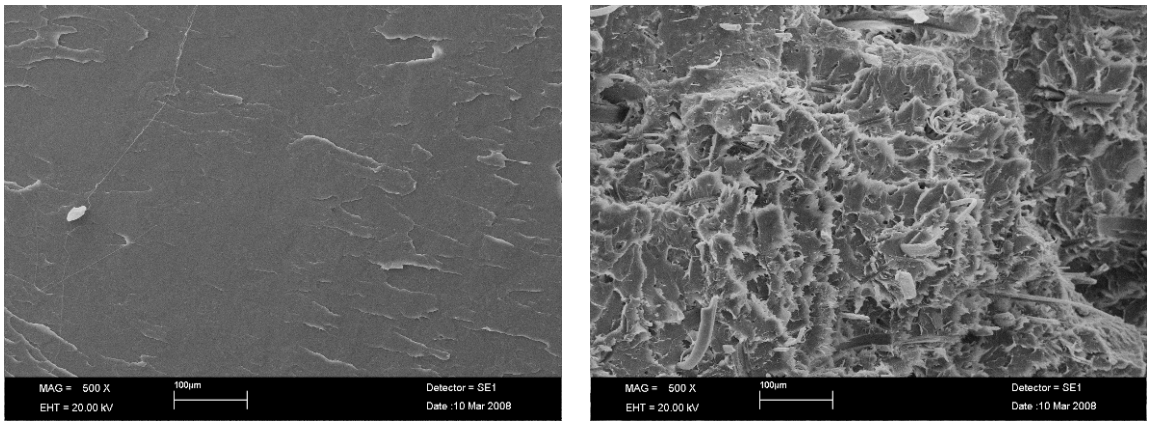


Fig. 5 SEM images of pure PLA samples (left) and silk/PLA biocomposites (right) after 8 weeks of biodegradation test.

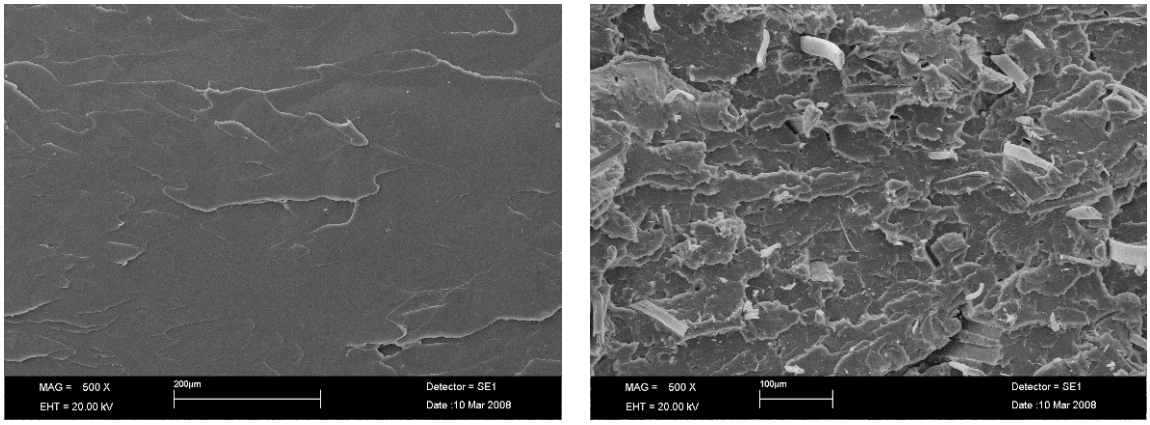


Fig. 6 SEM images of pure PLA samples (left) and silk/PLA biocomposites (right) after 12 weeks of biodegradation test.

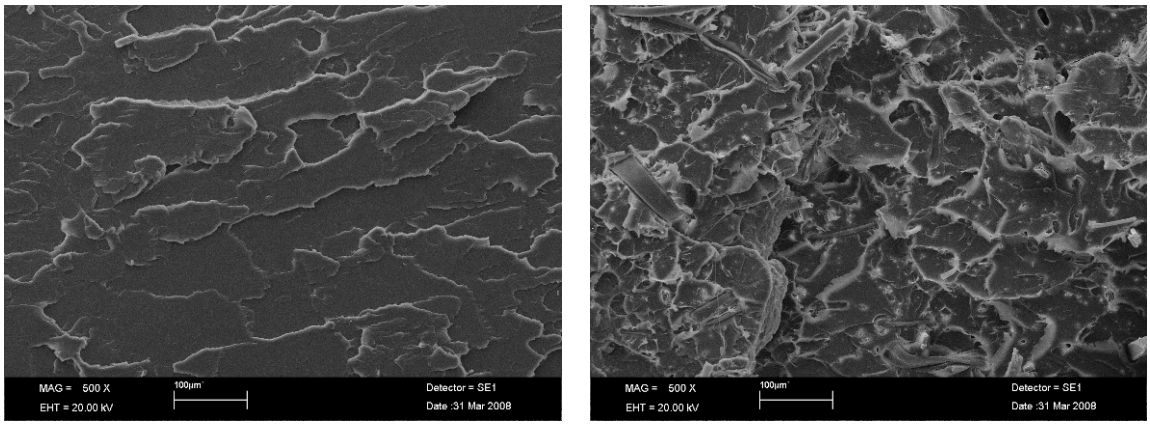


Fig. 7 SEM images of pure PLA samples (left) and silk/PLA biocomposites (right) after 16 weeks of biodegradation test.