Elimination of large particulate units from silk fibroin PLD films by post-treatments

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Abstract
After depositing silk fibroin (SF) thin films by pulsed IR-laser deposition, extraordinarily large particulate units up to several micrometers were observed. They include debris from the target and severely agglomerated protein units. Occurrence of those large particles was found to be minimum on the vertical substrate. We tried to eliminate large particulate units by two post-treatment operations, i.e. dry gaseous blow-off (GBO) and rinsing in water under simultaneous ultrasonication (WSU). Change in the surface structure by these post-treatments was observed by optical and electron microscopes with varying area from 1mm square down to \(1\ \mu\text{m}\) square. GBO turned out to be suitable to eliminate the lightly attached particulates of \(1-10\ \mu\text{m}\), mostly those pulled out from the target while preserving morphological and chemical properties of smallest units underneath. WSU, on the other hand, pelt off more strongly attached surface irregularities. However, morphological change with an increase in the surface roughness in the range of 1nm was also observed after WSU. The latter might be associated with possible sonochemical effects.

1. Introduction
Preparation of protein thin films via IR-based pulsed laser deposition (PLD) is an emerging technology. In our previous report [1], we have demonstrated the ablation of non-absorbable protein by near infrared nanosecond laser and evaluated the silk fibroin nanofilms. Depending on the application purpose of the films, it is desirable to control the surface roughness. Extraordinarily large particulate units on the film comprise debris due to direct pullout from the target and drops from the target as a consequence of partial melt down or local explosion.

Formation of those large units can be avoided to a substantial extent by optimising the deposition condition. The latter often requires, however, suppression of laser fluence (LF) and/or deposition time to restrict the rate or efficiency of the film deposition process [1]. It is, therefore, necessary to look for some post-treatment methods to make the film surface smoother. Purpose of this work is, therefore, to examine, whether and to what extent we can eliminate undesired large particulates by post treatments after PLD.

2. Experimental
Targets were prepared by compressing refined cocoon powder from \textit{Bombyx mori} (Idemitsu Petrochemical Inc. Protein Powder, average particle size \(7.9\ \mu\text{m}\)) at 80\(^\circ\text{C}\) and 10MPa for 30min. Fibroin was deposited on Si (100) by using Nara Laser Ablation System (Nara Machinery), equipped
with 1064nm Nd:YAG laser under the conditions; pulse width 5ns, pulse frequency 10Hz, and fluence between 2J/cm² and 5J/cm². The threshold value has been determined to be 1.7J/cm² [1]. Helium was used exclusively as a background gas at a fixed pressure, 100Pa. All the PLD processing was carried out at room temperature. Macroscopic inhomogeneity or locality of the occurrence of large particles was examined, by comparing the optical microscopic (OM) images of the film on the horizontal as well as the vertical substrates as schematically illustrated in Figure 1. The distance between the target and center of the horizontal (0,0) or vertical substrate was 20mm.

We tried two kinds of post-treatment techniques, i.e. (1) a dry process, gaseous blow-off (GBO), by using inert gas (He) with flow rates of 35.0 liter per minute, and (2) a wet process rinsing in water under simultaneous ultrasonication (WSU). For GBO, we have developed a blow orifice as shown in Figure 2. In a wet process, deposited film prepared by PLD was soaked into super deionized water, and treated by ultrasonic bath under a fixed condition, 28kHz, 250W for 5min, then rinsed with super deionized water and dried in 25°C.

Surface morphology in the wide view area, 225µm×150µm, was observed under a laser microscope (LM, Lasertec, LLM21). While we use an atomic force microscope (AFM, Vecco, Nanoscope IV) as a main tool for evaluation of morphology and microstructure of the film, a field emission scanning electron microscope (FE-SEM, HITACHI, S-4700) was also used. Effort was paid to quantify the surface morphology in terms of apparent surface roughness, \( R_{\text{rms}} \), obtained either from LM or AFM, with reference to the well-resolved structural observation by SEM.

3. Results

Occurrence of large particulate matters up to 50µm was observed on the film deposited at 2J/cm² for 0.5min, under the optical microscope. As shown in Figures 3, largest amount of large particles were deposited at the central position, (0,0) (Figure 3(a)) than other substrates in a horizontal position, while much fewer large particles are observed on the substrate in a vertical position, v1 (Figure 3(b)). When we observe the same film under AFM, we always observe the densely packed smallest protein units (SPU) in the majority of the substrate areas (Figures 3(c) and (d)). Note that the difference in the values of \( R_{\text{rms}} \) was insignificant, i.e., 0.394nm and 0.386nm, in the view area of 1µm×1µm for the position of (0,0) and v1, respectively.

We now observe the effects of GBO treatment on the films prepared under the same condition to those we observed in Figure 3, i.e. at 2J/cm² for 0.5min at the linear gas flow rate of 580 m s⁻¹, being maximum in our experiments. Elimination of larger particles by GBO treatment is obvious by comparing scanning electron micrographs shown in Figures 4(a) and (b), before and after GBO, respectively. We note that the root-mean-square surface roughness values, \( R_{\text{rms}} \), determined by laser microscope before and after GBO were 0.34µm and 0.30µm, so that the change in \( R_{\text{rms}} \) seems insignificant either. Note that extremely large particles often remain unblown, as shown in Figures 4(c) and (d). Blown off particles were captured and observed by SEM, as shown in Figure 5. We observe particles between 1µm and 10µm, corresponding to the size of intact SF powder.

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**Figure 1.** Scheme of the location of the substrates in the PLD chamber.

**Figure 2.** Schematic representation of the post treatments of gas blow-off (GBO).
For the same film, deposited at 2J/cm², we have exerted WSU treatment and observed under OM. As shown in Figures 6(a) and (b) before and after WSU, respectively, elimination of larger particulates between 10μm and 50μm was also visible. When we observed a thick film deposited for 60min (average thickness, 150nm), the value of \( R_{\text{rms}} \) decreased from 0.39μm to 0.16μm after WSU, as we determined by laser microscope. On the other hand, AFM observation of smaller view area of 1μm×1μm revealed the morphological change of the SPUs, as shown in Figure 6(c) and 6(d). The corresponding AFM-surface roughness increased from 0.394nm to 1.059nm.

Figure 3. Difference of the occurrence of large particulate units in the PLD film, prepared at 2J/cm² for 0.5min, depending on the location of the substrates observed under the optical microscope ((a) and (b)). (a) (0,0), horizontal and (b) vertical. (c) and (d) are AFM images corresponding to the optical micrographs of (a) and (b), respectively.

Figure 4. (a) and (b): Scanning electron micrographs of the PLD film deposited at 2J/cm² for 0.5min; (a) as deposited at (0,0) and (b) after GBO treatment for 5min. (c) and (d), OM images for the same samples as (a) and (b).

Figure 5. SEM image of the particles blown off, captured on the screen behind the blow-off set up.

Figure 6. Optical micrographs ((a) and (b)) and AFM images ((c) and (d)) of the film deposited at 2J/cm² for 0.5min at (0,0). Effects of WSU treatment are shown in Figures (b) and (d) as compared to as deposited films, (a) and (c).

4. Discussion
As mentioned above, GBO treatment was successful, but to a limited extent. Our blow-off linear flow rate, 580 m s⁻¹, is well above conventional upper limit of the practical classifier or conveying system [2-4]. While the available literature data are basically associated with the inertial force without adequately taking adhesive forces into account, the blow-off operation rests upon the fluid dynamics...
of particle entrainment [5], where theoretical treatment is restricted to the macroscopic area. Microscopic fluid dynamics is yet to be elucidated.

Another limitation of the post-treatment is the possible sonochemical effects during WUS operation, as we suspected from the morphological change in the units as small as ordinary SPU of fibroin, as shown in Figure 6(d). While a number of sonochemical reactions were reported in wide areas of materials [6,7], those on the protein species are restricted. While the ultrasonication brings about spheroidization of some proteins like avidin [8], its activity seems to be lost. In the case of human immunoglobulin, its primary structure was preserved during ultrasonication for the purpose of encapsulation, but a sign of partial denaturalization was observed [9]. While biological cells are partially broken by ultrasound, they are partially activated due presumably to the formation of radicals [10]. The post treatment WSU should, therefore, be carried out with care to meet the application purposes.

5. Conclusion
A post treatment technique, gas blow-off (GBO) turned out to be suitable to eliminate the lightly attached particulates of 1-10μm, while preserving morphological and chemical properties of SPUs between or underneath the large particles. Water rinsing under simultaneous ultrasonication (WSU), on the other hand, pelt off more strongly attached surface irregularities. However, morphological change with an increase in the surface roughness in the range of 1nm was also observed as a side effect. The latter might be associated with sonochemical effects. We therefore conclude, that, by an appropriate choice of the method, simple post-treatment operation on the fibroin films can bring about smoother surfaces either by gaseous or by aqueous processing, although care should be taken with possible sonochemical effects in case of WSU treatments. Conversely, given the particle size range and by exerting systematically varying linear air flow rate, we may be able to estimate the adhesive force of the particulates on the protein film.

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References
[1] Nakayama S, Nagare S and Senna M 2006, Thin Solid Films, in press
[2] de Boer AH, Hagedoorn P, Gjaltema D, Goede J, Frijlink H W, 2003, Int. J. Pharm. 260, 187
[3] McCallion, O N M, Taylor, K M G, Bridges, P A, Thomas M and Taylor A J, 1996, Int. J. Pharm. 130, 1
[4] Ji H, Tsutsumi A and Yoshida K, 1998, J. Chem. Eng. Jpn, 31, 842
[5] Phillips M, 1980, J. Phys. D: Appl. Phys. 13, 221
[6] Suslick K S, Price G J, 1999, Ann. Rev. Mater. Sci. 29, 295
[7] Torii T ; Yasui K, Yasuda K, ; Iida Y, Tuziuti T, Suzuki T, Nakamura M, 2004, Res. Chem. Intermediates, 30, 713
[8] Avivi S, Gedanken A, 2005, Ultrasonics Sonochem, 12, 405
[9] Wang J J, Chua K M, Wang C H, 2004, J. Colloid Interface Sci. 271, 92
[10] Bohm H, Anthony P, Garratt L C, Briarty L G, Lowe K C, Power J B, Benes E and Davey M R, 2002, Artificial Cells Blood Sustitutes Immobilization Biotechnol, 30, 127