Thin Solid Films 542 (2013) 214–218

Contents lists available at SciVerse ScienceDirect

Thin Solid Films

journal homepage: www.elsevier.com/locate/tsf

Smooth silk fibroin nanofilm deposited by 1064-nm pulsed laser beam from an opaque target

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ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form 5 July 2013
Accepted 9 July 2013
Available online 17 July 2013

Keywords:
Silk fibroin
Nanostructured thin film
Pulsed laser deposition
Multiple Mie scattering
Optical breakdown

ABSTRACT

In an attempt to prepare smooth nanostructured thin films of silk fibroin (SF) by near-infrared (NIR) pulsed laser deposition, an opaque target was prepared from an emulsified aqueous solution of SF. Upon irradiation of 1064-nm pulsed laser beam at its fluence 5 J/cm^2, a thin film of SF was deposited on the Si(100) substrate with its root-mean-square surface roughness, 0.37 nm, smoother than those obtained from a compressed target of SF powders by approximately an order of magnitude. The attainment of an extra-smooth film from the opaque target was discussed in terms of multiple Mie scattering of the incident NIR beam, leading to an increase in the plasma density, intensified optical breakdown, ablation of better dispersed SF molecular units, and a film with more intensive intermolecular cross-linking.

1. Introduction

Laser deposition is a versatile tool for thin film deposition of biocompatible polymers, preferentially via a matrix assisted pulsed laser evaporation technique [1–6]. A simpler pulsed laser deposition (PLD) by near-infrared (NIR) irradiation also enables formation of protein thin films [7,8]. Photon energy of 1064 nm Nd:YAG laser beam is 1.17 eV and hence significantly lower than any covalent bond energy, e.g. C-C: 3.6 eV, or C-N: 3.0 eV. Therefore, PLD with an NIR beam protects silk fibroin (SF) from structural degradation, as we demonstrated hitherto [9,10].

SF does not absorb 1.17 eV photons, so that usual photochemical or photothermal mechanisms do not work. Therefore, ablation is accomplished via avalanche ionization and associated optical breakdown [9]. As a matter of fact, similar mechanisms were working in the field of laser surgery [11,12]. In order to obtain uniformly nanostructured films by PLD, it is important to avoid formation of chunk or debris. One of the main mechanisms of chunk formation is particle pullout from the targets. This takes place seriously when target was prepared by consolidation of powdery materials [10]. As we used appropriate binders for better inter-particulate adhesion within a target, chunk formation was significantly suppressed [13,14]. However, the surface roughness of the film remained unimproved.

In the case of inorganic crystalline materials, it is well known to enhance epitaxy during growth of the film for higher smoothness of deposition films [15,16]. For organic macromolecules, in contrast, a microstructure of thin films is predominated by molecular interaction and resulted orientation [17,18]. Therefore, we explore methods of smoothening of SF PLD films to manipulate the structure of the target. An aqueous solution of SF was dispersed into a hydrophobic medium to obtain a W/O emulsion, from which we prepared an opaque target. By comparing its ablation processes with those from the previous target comprising compressed SF powders, as well as monolithic transparent ones, we here try to elucidate key factors to smoothen the SF thin films obtained from PLD. Smoother protein film deposition is particularly desirable for the antibacterial surface modification of microdevices such as biosensors and implants by tracking the sub-micrometric surface structure with high fidelity [19,20].

2. Experimental details

2.1. Preparation of SF solution

Degummed *Bombyx mori* silk was prepared by the methods we described elsewhere [14]. The extracted SF was dissolved in an aqueous 9.3 M LiBr (99 + %, Research Chemicals Ltd.) solution at 60 °C for 4 h, yielding a 20 w/v % SF solution. The SF solution was dialyzed in a cellulose tube against distilled water at 5 °C for 4 days by replacing the external water every 12 h to obtain a purified aqueous solution of SF, i.e. SF_{aq}. The final concentration of SF_{aq} was approximately 4.8 mg ml^{-1}, which was determined by weighing the remaining solid after drying at 50 °C for 12 h under ambient pressure.

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http://dx.doi.org/10.1016/j.tsf.2013.07.022

2.2. Preparation of SF targets

SF_{aq} was kept at 5 °C for 1 month to generate an opaque white colored hydrogel. After drying the hydrogel at 50 °C for 12 h, we obtained a transparent xerogel, which was used as a "xerogel target" for PLD. For the purpose of emulsification, the SF_{aq} was dispersed into cyclohexane with a nonionic surfactant, polyoxyethylene dodecyl ether (POD5, EMALEX 705, Nihon Emulsion Co., Ltd.). The volume ratio of SF_{aq}: cyclohexane: surfactant was kept constant at 1: 3: 0.005. The mixed solution was mildly stirred for 10 min at room temperature and then kept for 1 h in a draft chamber. The emulsion thus obtained was heated at 50 °C for 4 h for cyclohexane evaporation. The wet gel obtained was subjected to extrusion through a sieve mesh with its opening 840 μm to obtain granules after drying at 50 °C for 12 h. The granules of the diameter between 600 μm–840 μm were then put into a mold of 30 mm in diameter, compressed uniaxially at 100 MPa for 5 min to obtain an "opaque target."

2.3. Pulsed laser deposition

The preparation method of SF thin films by PLD was given elsewhere [13,14]. Briefly, deposition was performed on Si (100) wafer (Komatsu Electronic Metals, p-type, B doped) by using Nara Laser Ablation System (Nara Machinery), equipped with Q-switched Nd:YAG laser (1064 nm, full width at half maximum ~5 ns).

The pulse frequency was fixed at 10 Hz. Incident laser spot area was 0.6 mm, focused down by a concave lens. The distance between the target and center of the substrate was kept constant at 20 mm. Laser fluence was fixed at 5 J/cm^2. We chose this fluence, which was close to the doubled value of the threshold. The deposition time was also kept constant at 2 min. Helium was used as background gas with its pressure kept constant at 100 Pa.

2.4. Characterization

The size distribution of the colloidal units in SF_{aq} and the emulsion was determined by the dynamic light scattering (DLS) detector (Malvern HPPS). Microstructure and surface morphology of the film were observed under a field emission-scanning electron microscope (FE-SEM, S-4700, Hitachi) and an atomic force microscope (AFM, Nanoscope IV, Veeco). The values of the weight of the deposits were directly monitored by a quartz crystal microbalance (CRTM-6000, ULVAC) and converted to thickness by adopting the average apparent density of SF determined by pycnometry, 1.1 g/cm^3.

Particle size distributions and amount of debris in the films were quantified by an image analysis, using a software, Image J® (Image-Pro discovery, Enhanced Solution for Image Analysis). We evaluated the coverage with the aid of Image J. We defined percent area of debris, as the projected area occupied by particles larger than 2.4 μm, recognized by scanning electron micrographs, which is twice as large as the resolution limit, 1.2 μm/pixel. Details of the structural analyses were given elsewhere [13,14]. The surface morphology of the target was also observed under FE-SEM. The specimens were sputtered with osmium to prevent negative charge build-up.

3. Experimental results

3.1. Irradiation of NIR on the target

The xerogel target showed monolithic transparent structure as shown in Fig. 1a. As we irradiated NIR laser beam on the xerogel target, the incident beam went through the target and was absorbed by the backing stainless steel (Fig. 1b). The opaque target, on the other hand, looked optically heterogeneous (Fig. 1c). Upon irradiation under the same condition, a purple ablation plume was evolved from the target surface (Fig. 1d).

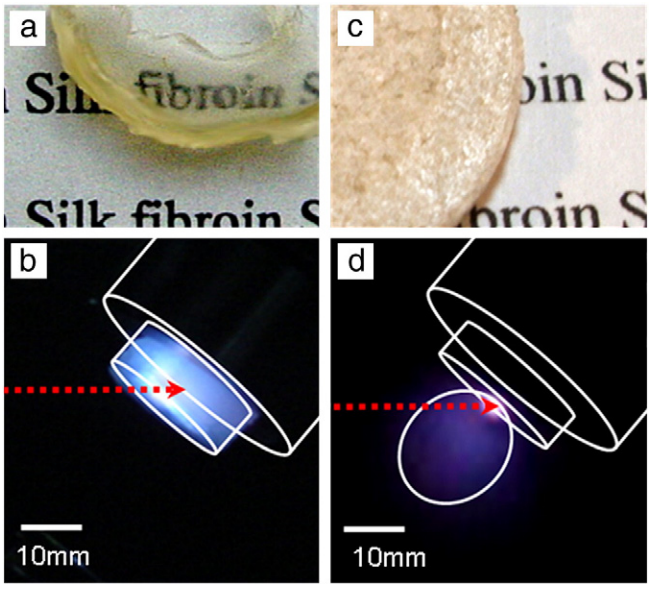

Fig. 1. Optical micrographs of xerogel target (a) and opaque target (c) with the state of plume during later irradiation at 5 J/cm^2 onto xerogel target (b) and opaque target (d).

Fig. 2 compares absorption spectra from the xerogel and opaque targets. One of the main features of the spectra is the absorption bands below 400 nm, ascribed to various amino acids [21], among others, glycine and alanine [22]. A small difference in these regions between two targets is presumably attributed to the change in the states of those amino acids due to agglomeration of globules, to be further discussed in 3.3. Note that the absorption at the wavelength of the incident beam, 1064 nm, remained negligible in both of the spectra, indicating no effects of POD5 emulsifier on the absorption of 1064 nm beam.

3.2. Dispersing units of SF in solution and emulsion

Dispersing units of SF in an aqueous solution and emulsion were examined by DLS. As shown in Fig. 3, the size distribution curves are bimodal, in both cases, peaked at 24 and 140 nm, and 22 and 240 nm for the aqueous solution and emulsion, respectively. The fraction of the latter units is much larger in the case of emulsion.

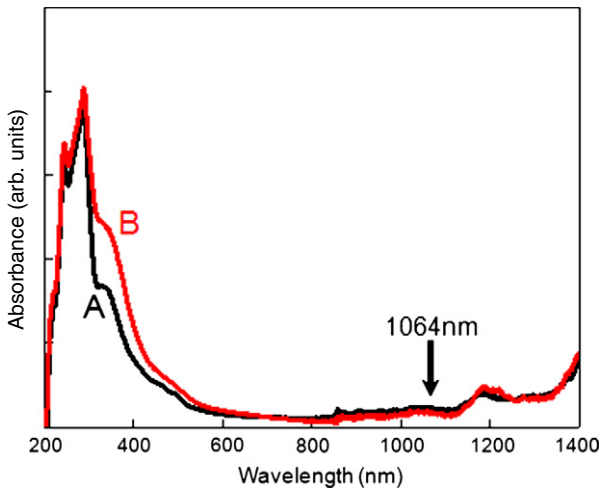

Fig. 2. Absorption spectra of xerogel target (A) and opaque target (B).