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A comparative study of the refractive index of silk protein thin films towards biomaterial based optical devices

A. Bucciarelli [a, *], V. Mulloni [b], D. Maniglio [a], R.K. Pal [c], V.K. Yadavalli [c], A. Motta [a], A. Quaranta [a]

[a] Department of Industrial Engineering, University of Trento, Via Sommarive 9, 38123 Trento, TN, Italy
[b] Micro System Technology Group, Fondazione Bruno Kessler, Via Sommarive 18, 38123, Trento, TN, Italy
[c] BioNano Laboratory, Department of Chemical and Life Science Engineering, Virginia Commonwealth University, 601 W. Main Street, Richmond, VA 23284-3018, USA

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ABSTRACT

Over the last two decades, silk fibroin has been exploited as a versatile optical material in biological applications due to a combination of unique properties. Recently, protocols have been developed to produce a silk fibroin negative tone resist that is UV crosslinkable, thereby allowing micro and nanoscale patterning of the protein using traditional photolithographic tools. The same protocol has been applied to the silk protein sericin to develop a sericin resist. Despite the immense potential of these biomaterials to develop micro optical patterns on silicon and glass surfaces, as well as self-standing components, their refractive indexes are not well characterized. In this work, optimizing a method to obtain extremely smooth, thin films, the refractive index (RI) of fibroin and sericin proteins and resists were characterized using ellipsometry. The parameters of the Sellmeier and Cauchy dispersion laws have been determined to obtain the RI over a large wavelength range. A complete morphological study of the films has been conducted. In addition, the effect of solvent on the optical properties of silk fibroin and sericin thin films are reported, with differences in values explained by examining the change in the protein secondary structure.

1. Introduction

For thousands of years, silks have been used for producing luxury textiles with exceptional texture, robustness, and shine. Over the last two decades, the two primary protein constituents of silkworm silk (silk fibroin and sericin) have been investigated by the scientific community for different technological applications, primarily tissue engineering and regenerative medicine [1,2], drug delivery [3–5] and, more generally, in the biomedical field [6,7]. Recently, the unique combination of properties of the silk protein fibroin - [8,9] viz. high transparency, mechanical robustness [10], and the possibility to be patterned at multiple length scales, has led to the development of several bio-optical devices, spanning from waveguides [11–13] to sensors [13–16]. The other bioproduct of silkworm cocoons, sericin, has also been used in many biomedical applications [17–20]. However, to date, due to the yellowish color of thick (more than 10 μm) free-standing films typically formed, sericin has not been suitable for applications in the fabrication of optical systems. In addition to interesting optical properties, the biocompatibility and biodegradability of these proteins provide unique options towards the fabrication of multifunctional optical devices that can be used in physiological microenvironments [21].

In recent years, the development of silk protein based UV-crosslinkable materials has further expanded their use. To obtain UV sensitive layers, methacrylate groups have been grafted to the protein amino acids through a conjugation reaction, resulting in protein “photoresists” that behave as negative tone materials. This procedure allows the synthesis of both fibroin photoresist (FPP) [22], and sericin photoresist (SPP) [23] for the realization of photocrosslinkable silk protein micropatterns on silicon and glass surfaces, as well as for the production of free-standing, flexible micro-optical components [24]. Recently, using the same modification procedure on light chain fibroin (LC fibroin) a more defined resist has been developed [25], allowing the formation of patterns with a resolution of 1.51 μm and a roughness of 2.3 nm. These

* Corresponding author.
E-mail address: Alessio.bucciarelli@unitn.it (A. Bucciarelli).

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materials pave the way to realize new, microfabricated, protein-based, biocompatible optical devices.

One of the most important parameters for the design of optical devices is the refractive index (RI). To date, in nearly all literature reports, the RI of silk fibroin has been characterized at single wavelengths, namely 630 nm [11,12] and 500 nm [26] giving a value around 1.54 and 1.52 (at 80% Relative Humidity) respectively. Since this RI is higher than that of soda-lime glass (RI = 1.5), it is possible to fabricate optical waveguides [11] with fibroin coatings on glass substrates. A core-cladding waveguide has been fabricated by exploiting the RI difference between two different forms of silk fibroin: using a hydrogel for the cladding (RI = 1.34) and the solid form for the core (RI = 1.54) [12]. An attempt to produce silk fibroin based optical fibers right after the degumming process has been conducted, but the numerical evaluation of RI was not reported [27]. The RI change due to electron [28] and γ [29] irradiation of silk fibroin self-standing films has been investigated using UV–Vis spectroscopy, a methodology commonly used for thick samples. In this case, the entire RI trend over the visible spectrum was calculated, but the data at 630 nm was observed to be inconsistent with previous works. This inconsistency probably arises from the method used, exploiting reflectance and transmittance measurements, which provides reliable results only in the case of samples with perfectly flat surfaces, typically not achievable on self-standing fibroin films. Only recently, ellipsometric analyses over a broad spectral range (250–1750 nm) have been performed on thin films of titania – silk fibroin nanocomposites [30]. These have been used in combination with pure fibroin thin films to develop an alternating stack usable as a colorimetric humidity sensor [26]. Similar RI measurements have also been conducted on fibroin films from different silkworm species [31]. A detailed analysis on the refractive index of coatings a few microns thick, produced with water-based fibroin was reported, providing evidence on how the RI value does not depend on the cocoon degumming procedure, and how it is higher in β-crystalline structures.

Despite these reports, detailed investigations are still needed to clarify the optical properties of these biomaterials. Up to now, only materials produced starting from water-based silk fibroin solutions have been used, excluding alternative solvents that could be beneficial for the fabrication process but which, together, could affect the fibroin secondary structure and, consequently, the refractive index [32]. For example, this is the case in the use of formic acid (FA), a benign solvent, which leads to the formation of a different crystalline compact secondary structure in films obtained by casting. We have earlier reported a method to obtain thin films of high optical quality based on spin coating using formic acid as the solvent of choice [33]. This method allows the production of exceptionally flat and uniform silk protein films with high transparency, in comparison to films obtained using either water or hexafluoroisopropanol (HFIP), both commonly used solvent systems. The RI of the films measured at 633 nm (1.558) was slightly higher than the RI reported earlier for silk fibroin films (1.54).

The refractive index of sericin itself has not been characterized so far, given the formation of yellow films as stated above. Owing to the utility of the recently synthesized UV-crosslinkable silk proteins (FPP and SPP) in microfabrication, understanding their optical properties is also of great interest. In this work, spin coating techniques have been optimized in order to obtain thin protein films with extremely low surface roughness over the nano and micro-scales. The RIs of fibroin, sericin, FPP and SPP cast using formic acid were measured by spectroscopic ellipsometry. In addition, the effect of solvent was studied by comparing thin films of fibroin cast from water and formic acid. A RI increment of FPP with respect to that of water-based fibroin was revealed, while the modification of silk sericin decreased its RI in comparison to pure sericin. Following UV treatment, FPP films showed a slight increment of RI. In contrast, SPP films exhibited no RI variation regardless of UV crosslinking. These results can be used to guide the design and fabrication of biofriendly optical devices using a toolbox of silk proteins namely, fibroin, sericin and their UV-crosslinkable variants – FPP and SPP.

2. Materials and methods

2.1. FPP and SPP synthesis

The extraction and purification of silk fibroin from silkworm cocoons was conducted following a well-established protocol [34]. A method previously described was used to synthesize FPP and SPP [22,23]. Briefly, lyophilized fibroin was dissolved in a 1 M solution of Lithium Chloride (LiCl, Sigma Aldrich) in Dimethyl Sulfoxide (DMSO, Sigma Aldrich) under N_2 flux at the controlled temperature of 60 °C until the complete dissolution. A stoichiometric quantity of 2-isocyanoethyl methacrylate (IEM, Sigma Aldrich) was added to the solution to allow the conjugation reaction. After 4 h of reaction under stirring at 60 °C, cold ethanol was added to precipitate the functionalized protein. A washing and centrifuging procedure was repeated 3 times using a cold mixture of 50% acetone (Fisher Scientific) and 50% absolute ethanol (EtOH, Carlo Erba Reagents). The collected product was lyophilized for 48 h to obtain a yellowish powder. The same process, using a stoichiometric quantity of IEM in the reaction, was used to obtain the SPP powder from pure sericin (Wako Chemicals).

2.2. Solutions preparation

To obtain thin films suitable for ellipsometric analyses by spin coating, a low concentration of protein in solution is a preliminary requirement. This method was optimized using protein resist (FPP/SPP) solutions with a concentration of 1.5 wt% in formic acid (FA) (FPP/FA and SPP/FA). Aqueous solution of silk fibroin (regenerated silk fibroin (rSF)/H_2O) was obtained by dilution of a 5 wt% water solution to 2%, obtained following the protocol described elsewhere [34]. FA solutions of silk fibroin (rSF/FA) and sericin (SN/FA) were obtained by dissolving 2 wt% of lyophilized silk fibroin and 2 wt% of pure sericin in FA respectively. The concentrations of rSF/H_2O, rSF/FA, and SN/FA solutions were higher with respect to FPP/SA and SPP/FA in order to obtain reference films with a thickness comparable with silk protein resist films.

2.3. Substrate preparation

Protein thin films were deposited on silicon substrates to perform ellipsometric measurements using the previously developed method [33]. To allow a good protein adhesion and a better thickness uniformity, surfaces were functionalized by means of an acrylate compound. The initial surface cleaning was conducted for 1 h in ambient condition with a piranha solution (3 part of H_2SO_4 98% Sigma Aldrich, 1 part of H_2O_2 30% Sigma Aldrich). Subsequently, cleaned surfaces were functionalized by exposing them to 3-(trichlorosilyl) propyl methacrylate (TPM, Sigma Aldrich) in a desiccator for 14 h under vacuum. To eliminate the excess of deposited TPM, treated surfaces were washed in hexane and water. Glass slides for transmittance spectra were polished in a piranha solution for 1 h in ambient condition and then washed with water and ethanol and dried at 180 °C for few seconds.

2.4. Film preparation

The film deposition process was conducted inside a clean