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Naturally functionalized silk as useful material for photonic applications

Susanna Cavallini [a], Stefano Toffanin [a,*], Camilla Chieco [b], Anna Sagnella [c], Francesco Formaggio [a], Assunta Pistone [c], Tamara Posati [d], Marco Natali [a], Marco Caprini [a,e], Valentina Benfenati [c], Nicola Di Virgilio [b], Giampiero Ruani [a], Michele Muccini [a], Roberto Zamboni [b], Federica Rossi [b]

[a] Istituto per lo Studio dei Materiali Nanostrutturati, Consiglio Nazionale delle Ricerche, Via Gobetti 101, 40129 Bologna, Italy
[b] Istituto di Biometeorologia, Consiglio Nazionale delle Ricerche, Via Gobetti 101, 40129 Bologna, Italy
[c] Istituto per la Sintesi Organica e la Fotoreattività, Consiglio Nazionale delle Ricerche, Via Gobetti 101, 40129 Bologna, Italy
[d] Laboratory MIST E-R, via P. Gobetti 101, 40129 Bologna, Italy
[e] Department of Human and General Physiology, via S. Donato 19/2, University of Bologna, 40127 Bologna, Italy

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ABSTRACT

Silk is a natural fibre obtained from the *Bombyx mori* silkworm cocoons that can be used in a wide range of fields thanks to its inherent multifunctionality.

Post-production steps are necessary to impart colour to the fibres to employ the material for optics and photonic applications, such as in fluorescence-based optofluidic devices in lab-on-a-chip realization.

Here we present an intrinsically greener dyeing approach for fabricating naturally functionalized silk, where highly-fluorescent organic dyes with lasing properties are *in vivo* up-taken by silkworms once introduced in the artificial diet. A detailed photoluminescence spectroscopy investigation is implemented to test whether the dyes are effectively incorporated within the silk proteins, in correlation with the silkworm gland positions where proteins extraction is held. Light amplification characteristics are demonstrated in silk extracted from glands of silkworm fed with artificial diet doped with Rhodamine B dye.

1. Introduction

Organic materials offer interesting properties as ultrafast non linear optical response, electronic and photonic multifunctionality, compatibility with a variety of technological platforms, large active area, mechanical flexibility and low-cost fabrication [1]. Moreover, the ability of organic molecules and polymers to provide efficient lasing in the solid state and the compatibility of organics with natural biomaterials makes organic photonics suitable to develop biocompatible and biodegradable devices such as organic lasers [2,3].

A material that has recently emerged as a highly promising candidate for this photonic application is silk fibroin, a widely available natural protein fibre possessing excellent mechanical and optical properties together with biocompatibility, biodegradability and implant ability.

Silk fibroin is a protein polymer harvested from *Bombyx mori* silkworm cocoons and assembled into a spectrum of material formats using a variety of fabrication techniques. The silk fibroin is present within *B. mori* silkworm cocoons as a double-stranded fibre, which is coated with glue-like proteins called sericins. The cocoon is composed of about 20–30% sericin and 70–80% silk fibroin and trace amounts of waxes and carbohydrates [4].

An easily implementable purification process of the silkworm cocoon enables to remove sericin which prevents the solubilization of the fibres, thus obtaining a regenerate silk fibroin water solution (RSF) that can be processed into various form: gels, sponges, blocks, foam and films [5].

Silk fibroin secreted in the lumen of posterior silk gland (PSG) of *B. mori* consists of three protein component: High (H)-chain 350 kDa, Light (L)-chain 26 kDa, and Glycoprotein P25 30 kDa which are connected by a disulfide linkage [6]. Silk fibroin can exist as three structural morphologies termed silk I, silk II, and III where silk I is a water soluble form and silk II is an insoluble form consisting of extended β-sheet. The silk III structure is helical and is observed at the air–water interface [7]. Among these various possibilities, films result very attractive for photonic applications thanks to their transparency, uniformity, surface flatness and capability of silk to replicate patterned substrate feature down in the nanometer range [8].

We have already demonstrated that a thin-film obtained by blending RSF with a suitable organic dye is able of lasing action once deposited on top of an one-dimensional photonic structure

* Corresponding author.
E-mail addresses: s.toffanin@bo.ismn.cnr.it (S. Toffanin), v.benfenati@isof.cnr.it (V. Benfenati), n.divirgilio@ibimet.cnr.it (N. Di Virgilio).

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[9]. The lasing threshold of this kind of structure is two order of magnitude lower with respect to other biocompatible distributed feedback structure (DFB) lasers [10].

The dyeing process which is necessary to modify silk optical properties in order to obtain lasing emission from the biomaterial involves several steps of post-treatment of silk solution.

Here, we present a multidisciplinary study for obtaining optically active silk substrate directly by feeding larvae of *B. mori* with specific dye molecules. This is a greener method to fabricate intrinsically coloured silk because it eliminates the need of resources as water, energy and post-processing modifications with the use of organic solvents. We performed silk proteins (fibroin and sericin) extraction directly from the silkworm glands before spinning in order to have the complete control of the doping procedure. In Fig. 1 we report the picture of the naturally-coloured silk cocoons after organic dyes are added into the standard silkworm diet.

In perspective, the final aim of the work is the fabrication of DFB structures for lasing implementing the intrinsically doped optically-active fibroin thin-film. In turn, the implementation of this device will allow the realization of high throughput low-cost lab-on-a-chip optofluidic device apt to fluorescence detection based on silk fibroin. Photoluminescence (PL) spectroscopy investigation is used as a powerful tool for discriminating the effectiveness of the biological functionalization of silk fibroin along the entire route: from the modification of the diet, to the extraction of proteins from different location of the silkworm glands, to the production of regenerate silk fibroin solution. Indeed, the organic conjugated dyes are typically very luminescent moiety, whose spectral features are intrinsically correlated to the chemical and physical interactions with the dispersing matrix.

2. Materials and methods

2.1. Modified feed experiments

The white cocoon polihybrid strain coming from germplasm collection of the CRA-API (CRA, Honey bee and Silkworm Research Unit, Padua seat) was used for breeding.

The silkworm were reared in controlled conditions [11] and the larvae were fed "*ad libitum*" with artificial diet also obtained by Sericulture Specialized Unit of Padua of the Experiment Institute for Agrarian Zoology [11] until 2nd day of 5th instar.

On the 3rd day of the 5th instar to the spinning of the cocoons the larvae were fed with a modified diet. The dye-added diet was prepared by mixing a known quantity of dye into the standard diet. The dye molecules we tested were Stilbene 420, at the concentration of 0.5 gr and 1 gr in 100 gr of powder diet respectively and Rhodamine B, at the concentration of 0.05 gr in 100 gr of powder diet according with Tansil et al. [12].

Fig. 1. *Bombyx mori* cocoons naturally functionalized with different dyes (Rhodamine B, Nile blue and Stilbene 420).

Stilbene-added diet was administered to 25 larvae for stilbene 0.5 gr and to 25 larvae for stilbene 1 gr; the Rhodamine B-added diet was used to feed 50 larvae.

2.2. Native silk fibroin extraction

Native silk fibroin (NSF) was extracted from the glands of 5th instar larvae before spinning; the fibroin extraction from the middle division of silk gland (MSG) was performed according to Hossain et al. [13] with a partially modified protocol, while the posterior parts of the gland (PSG) were treated according to Mandal and Kundu [14]. In brief, the entire silk glands were pulled out from the abdominal side of the worm and the middle part was separated from the posterior part. The middle glands were washed in deionized water and the surrounding epithelium was gently removed; the glands were immersed in 3 ml distilled water to remove most insoluble sericin protein. After 6 hours the water was removed and other 3 ml of distilled water were added and the solution was maintained at 5 °C until the total dissolution of fibroin; then the solution was collected in a falcon tube and stored in refrigerator. The posterior glands were washed with distilled water to remove traces of sericin and placed into a beaker containing 3 ml of distilled water; the glandular tubes were cut in small pieces and gentle shaking for 1 hours, then kept in refrigerator overnight. After one day the protein released from the glandular tissues was collected in a falcon tube and stored in refrigerator.

2.3. Regenerated silk fibroin water-solutions

Regenerated silk fibroin (RSF) water-solutions were prepared from silkworm white and coloured cocoons (Istituto di Biometereologia, CNR of Bologna) according to the procedures described in our previous studies [15,16]. The cocoons were degummed in boiling 0.02 M Na_2CO_3 (Sigma–Aldrich, St. Louis, Mo) solution for 45 minutes. The SF fibres were then rinsed three times in Milli-Q water and dissolved in a 9.3 M LiBr solution at 60 °C for 6 h. The SF water-solutions were subsequently dialyzed (dialysis membranes, MWCO 3500) against distilled water for 48 hours and centrifuged to obtain pure regenerated SF solutions (ca. 4 wt/vol%). The SF water-solutions were stored at 4 °C.

As reference in the optical spectroscopy characterization, we used a solution obtained from blending RSF water solution with Stilbene 420 at 5% in weight.

2.4. Optical spectroscopy characterization

The photoluminescence (PL) spectra of stilbene-intrinsic doped solutions were collected exciting the samples with a He:Ne laser at 325 nm in transmission configuration. The fluorescence spectra were collected using an Optical Multi-channel Analyzer (Hamamatsu). PL Quantum yield measurements of solutions were carried out inside a 6-in. integrating sphere as described elsewhere [17], implementing a diode-laser at 375 nm as excitation source in the case of the functionalization with stilbene dye, whether a 440 nm He:Ne laser source was used to excite the Rhodamine B naturally-doped silk (so that silk samples obtained from larvae fed with dye added diet).

ASE measurements were performed on a 12 μm-thick drop-casted film of Rhodamine B naturally-doped silk placed in a vacuum chamber (10^{-6} mbar) and pumped with a Nd:Yag laser at 532 nm; the excitation area was a (200 μm × 2 mm) stripe that hit the sample perpendicularly to its substrate, whereas the emitted spectra was collected parallel to the substrate.