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Bio-doping of regenerated silk fibroin solution and films: a green route for biomanufacturing

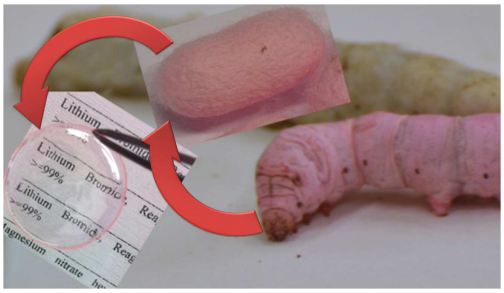
A. Sagnella, [†a] C. Chieco[†b], N. Di Virgilio[b]*, S. Toffanin[c], T. Posati[a], A. Pistone[d], S. Bonetti[c], M. Muccini[c], G. Ruani [c]*, V. Benfenati[d]*, F. Rossi[b] and R. Zamboni[d].

[a] Laboratorio di Micro e Submicro Tecnologie abilitanti dell'Emilia-Romagna (MIST ER), Via P. Gobetti 101, I-40129 Bologna, Italy

[b] Consiglio Nazionale delle Ricerche- Istituto di Biometeorologia (CNR-IBIMET), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: n.divirgilio@isof.cnr.it; Tel: +39-051-6399004

[c] Consiglio Nazionale delle Ricerche-Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: g.ruani@bo.ismn.cnr.it; Tel: +39-051-6398508

[d] Consiglio Nazionale delle Ricerche – Istituto per la Sintesi Organica e la Fotoreattività (CNR-ISOF), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: Valentina.benfenati@isof.cnr.it; Tel: +39-051-6399760



Silk fibroin optically active films could be successfully obtained by biodoping i.e. from cocoons of *Bombyx mori* fed with Rhodamine B added diet.

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Bio-doping of regenerated silk fibroin solution and films: a green route for biomanufacturing

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Silk fibroin (SF) is a natural biocompatible material that can be integrated in a variety of photonic systems and optoelectronics: i.e. organic lasing from dye doped nano-structured silk film. In this context, biological incorporation of doping molecules into SF by means of feeding silk worms with addition of dyes to the diet, could be an innovative and eco-sustainable approach to obtain doped SF substrates, that avoids additional chemical processes and post-treatments of protein solution. In the present work, we demonstrated that SF regenerated solutions and films containing Rhodamine B (RhB) could be successfully obtained from the cocoons of *Bombyx mori* fed with RhB added diet (RhB-md-SF). Comparative analyses of optical and vibration characteristics of RhB-md-SF solution and films with those of white SF blended with RhB (RhB-d-SF) revealed significant difference, suggesting that silkworms metabolism could be involved in binding mechanism of SF with RhB.

In conclusion, we observed that doping diet is a promising method for green fabrication of SF-based optically active materials opening novel routes for silk-based biophotonics.

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Introduction

Recent progress both in technological research and manufacturing, as well as depletion of non-renewable raw material resources rise interest in the need of engineering alternatives for the development of a sustainable, eco-friendly material platform and material processing that can be used for manufacturing of the future. In this context, one of the most challenging issue is to identify a material that satisfies either technological and sustainability features. Among different biopolymers, the recent research on silk fibroin from the silkworm *Bombyx mori* (Linnaeus, 1758) highlights its potential as a sustainable, biocompatible, biodegradable and technological material, that can pave the way towards the replacement of plastic or oil-based materials in existing photonic and electronic device platforms as well as in device intended for biomedical purposes.[1-3]

Silk is a well-known natural fibre produced by *B.mori* which has been used for centuries in form of threads. Besides the very popular use of silk threads for biomedical application, recently interest has been increasing in the use of so-called regenerated silk fibroin (RSF) water-solution, to obtain bio-derived materials for technological[1-2] and biomedical applications.[4-5]

Silk fibroin substrates and materials from regenerated solution displayed several remarkable and well-documented characteristics, including mechanical flexibility, controllable biodegradability, biocompatibility, dielectric properties and capability of drug/doping inclusion, stabilization and release.[1-5] It should be noted that silk could be regenerated into a desirable form as sponge, hydrogel and film, to meet a specific application.

Among different forms of SF-based biomaterials, SF-films have recently found increasing applications in tissue-engineering, including the generation of artificial nerve guides for peripheral nerve repair.[6] Feasibility of using SF materials obtained from regenerated solution for opto-electronic biomedical application has been demonstrated *in vitro* and *in vivo*.[6]

In particular, we showed that silk can be moulded onto patterned substrates with features down to tens of nanometers for obtaining mechanically stable structures with optical wavelength size.[7] We have demonstrated organic lasing from a blue-emitting stilbene-doped silk film spin-coated onto a one-dimensional distributed feedback grating (DFB). The lasing threshold is lower than that of organic DFB lasers based on the same active dye on the grating. More recently, modified DFB laser based on Rhodamine doped SF-films with scattering particles randomly distributed, has been demonstrated[7a].

These findings pave the way to the development of an optically active biocompatible technological platform based on silk.

Several processes have been reported to dope SF with optically active molecules, growth factors and/or chemicals.[5]

However, some chemical and physical post-processing treatments of SF could damage/denature the protein modifying completely its primary properties, and in turn the properties of the films. Indeed, in the silk film, the polypeptide chains of fibroin protein self-assemble in the secondary structures that determine the formation of two different protein conformations: the silk I (water-soluble), rich in α-helix and random coils, and the silk II (water-insoluble), rich in β-sheets.[8] The balance between the dominance of α-helix and random coils with respect to β-sheets secondary structures determine the chemical-physical and mechanical properties of the substrates. Therefore, all processes to which the SF is subject should be highly controlled and, in some cases, limited.

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The biological incorporation of doping (drug) molecule into SF by means of feeding larvae is an eco-sustainable method of producing doped, optically active silk substrates because it eliminates the need for an external chemical process of the solution, and post-treatments associated with it.

In this view, recently a series of fluorescent dyes have successfully been incorporated in silk fibre by addition of colorant compounds to the silkworm diet following protocols previously described.[9] In particular, Tansil et al. mixed Rhodamine B (RhB) into mulberry powder at different concentrations to make modified feed for the silk worms at three days of fifth instar. They showed that successful formation of pink and luminescent cocoons and silk threads could be obtained with different intensity of colour and luminescence depending on the concentration of RhB in the diet. Of more importance, the authors observed that RhB could be detected in the core of fibroin fibres that were luminescent under UV irradiation, and not only externally in the sericine. The body of silkworms and the fibres assumed a pink colour and the biodistribution study of xenobiotic in *B. mori* revealed an uptake of RhB in the native fibroin solution of the protoracic gland.[9]

However, a study regarding adequate extraction and preparation of the regenerated silk fibroin solution from the modified diet cocoon, as well as a detailed control of chemo-physical properties assessment of silk fibroin films extracted from the diet modified cocoon is lacking while it is essential to translate and validate the method for silk-based technological manufacturing. This is not so trivial as the several passages of the extraction and purification processes could compromise efficient inclusion of the doping molecules in the final film substrates.

In order to establish *B. mori* doping diet as a natural method for biomanufacturing of optically active silk-based technological materials, here we study the chemo-physical properties of regenerated silk fibroin solution and film extracted from cocoon obtained by RhB modified diet method described by Tansil et al..[9]

We show that optically active silk fibroin solution and films can be obtained by RhB modified diet cocoon. The features of absorption and emission spectra of solution and films resemble those of RhB, and are compared to those obtained applying common method of addition of doping molecule to white silk fibroin solution. FT-IR analyses revealed that white and RhB-md-SF films displayed the same fingerprint confirming that the self-assembly capability of SF protein was not affected. Collectively, our results open the view for the use of diet doping method as novel biomanufacturing process for silk based technological substrates for eco-sustainable optoelectronic and photonics.

Experimental

Silkworm rearing. The insect breeding started from eggs belonging to the germplasm collection of the CRA-API (CRA, Honey bee and Silkworm Research Unit, Padua seat) which also provided artificial diet. The strain chosen for the experiment was the polyhybrid with white oval cocoons. Rearing was carried out in plastic boxes (different in size according to the larvae age) placed in a chamber under controlled conditions (temperature was set at 25 ± 1°C; relative humidity 70% and 12:12 L:D photoperiod). During the first fours the larvae were fed *ad libidum* with artificial diet,[10] while a weighted amount of diet was given daily during the fifth instar. [10] According to the reference,[9] RhB was mixed into artificial diet at a concentration of 0.05 wt% to make modified feed that was then fed to silkworms starting from the third day of the fifth instar. For groups of white strain, composed of twenty last instar larvae each, were put in a separated box and fed with modified diet starting respectively from the third (group A), fourth (group B) and fifth (group C) day of

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the fifth instar until the cocoon spinning. In this way we obtained different add-eat time cocoons, respectively of 96, 72, 48 and 24 hours. At the same manner, three groups of fifth instar larvae of the same strains were separatedand fed with the same diet except for the addition of RhB powder, and white cocoons were used as control.

Colorimetry. Cocoon colours components were collected and quantitatively measured on both cocoon sides (cheeks)by using CR-400 colorimeter (Minolta, Tokyo, Japan).

Preparation of regenerated SF water-solutions. Regenerated SF water-solutions were prepared from Bombyx mori silkworm white and natural coloured cocoons (CNR-IBIMET, Bologna) according to the procedures described in our previously studies.[3] The cocoons were degummed in boiling 0.02 M Na_2CO_3 (Sigma-Aldrich, St Louis, Mo) solution for 45 min. 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 h and centrifuged to obtain pure regenerated SF solutions (ca. 4 wt/vol %). The SF water-solutions were stored at 4 °C.

Formation of RhB-doped SF solution and RhB water-solutions. In order to value the optical properties of dye in different conditions we added the RhB in the white regenerated SF water-solution (produced by bred no dye-containing diet) and we also dissolved it in water. The concentrations of dye in the dispersions and solutions samples were comparable to those in the natural coloured SF water-solutions.

UV-VIS spectroscopy. A Jasco V-550 UV-VIS Spectrophotometer was used in order to obtain the UV-VIS absorption spectra from the protein solutions and the percentage transmittance from the SF films. These were prepared by casting a 40 µl aliquot of SF solution on quartz substrate (area of 1,44 cm^2) and then left to dry in a sterile hood. The thickness of the film was measured by a profilometer KLA Tencor, P-6 and it resulted around 20 µm. The fluorescence properties of SF solutions and films were collected by using a Spex 1934D Phosphorimeter. The emission profiles were carried out by exciting the samples at 325, 546, 274 and 295 nm.

Fourier Transform Infrared Spectroscopy. Absorption spectra have been performed on SF films casted on infrared transparent substrates (KBr single crystals) by means of a FT-IR Bruker IFS113v interferometer. The curve fitting of overlapping bands of the infrared spectra covering the amide I and II regions (1500-1700 cm^{-1}) were performed by using the Levenberg-Marquardt algorithm implemented in the OPUS 2.0 software.

Results and discussions

Colorimetric analysis of RhB modified cocoons and regenerated SF solutions.

The first step of our study was to feed polyhybrid strain of B. mori (Figure 1a) with RhB following the protocol of Tansil et al.[9] In particular, RhB was mixed into powder artificial diet at a concentration of 0.05 wt% to make modified feed that was then fed to silkworms starting from the third day of the fifth instar (Figure 1b).

To monitor the correlation of RhB inclusion in the cocoon with the time of exposure to the diet, 3 groups of white strains, composed of twenty last instar larvae each, were put in a separated box and fed with modified diet starting respectively from the third, fourth and fifth day of the fifth instar until the cocoon spinning. In this way we obtained different add-eat time

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cocoons, respectively of 24, 48 and 72 hours (Figure 1d-f).

Since silkworm fifth larval stage takes in about six days, to obtain a different exposure time to dye, the doped diet was administered on different days from the last molting, respectively from the third, fourth and fifth day up to the cocoon spinning.

At the same manner, three groups of the same strains fifth instar larvae were separated and fed with the same diet except for the addition of RhB powder, and white cocoons were used as control (Figure 1c).

Accordingly to the observation of Tansil et al. [9], the body of worm, feed with RhB modified diet, became coloured after 2-3 hrs from RhB diet assumption.

Aside from the colour, no difference (i.e. morphology and weight) was observed between the coloured and white cocoons that were produced by silkworms consuming normal non-modified feed.

Cocoon colours components were collected and quantitatively measuredon both sides.

The colour of internal and external side of cocoons were not significant and generally followed the same trend for all colour components. Thus, values obtained from internal and external side for each cocoon were grouped together.

Table 1 summarizes colour components for each time course treatment.

We found that Lightness (L) value ranged from 95.14 of the white SF to 89.22, following a decreasing trend. Value of Chromaticity (C) increased with time, from 9.83 to 25.35, while hue (h) values decreased, from 124.18° to 27.90° in the case of the 72h of feeding with RhB doped diet, which also resulted in the higher variation range. The 72h treatment resulted in a low decrement of h, while previous treatment significantly affected h value. H° value moved from 124.18° to 27.90°, then from in between of bluish-green and yellow component to almost red-purple component. The a colour component significantly increased, from a value of -5.51 to a maximum of 28.10, confirming the increment of the red-purple hue component with time.The reported data confirmed that the increased time of exposure to RhB diet promote increase of the red-purple hue component in the cocoon colour.

These data suggest that an increase in colour can be obtained depending on the time of exposure to RhB-modified diet.

According to Tansil et al.[9], both the silkworm body and the resulting silk cocoon were also highly luminescent, appearing orange under UV irradiation, suggesting the inclusion of fluorescent molecule.

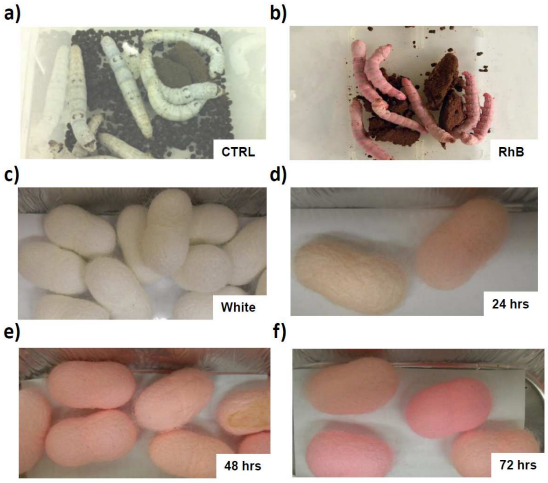

Figure 1. Larvae of *Bombyx mori* domesticated on a) standard diet no modified and b) doped diet with RhB. c) Cocoons from silkworms of *Bombyx mori* fed with no doped diet. d-f) Natural coloured cocoons from silkworms fed with diet containing dye. Different rose shades were due to time of exposure of larvae to diet (24, 48, 72 hours).

Time (h)	N	L	C	h°	a	b
0	2	95.14	9.83	124.18	-5.51	8.13
24	8	91.22	17.64	66.74	6.91	16.02
48	8	87.61	27.20	30.94	23.39	13.49
72	12	87.96	31.85	27.90	28.10	14.29
tot	30	89.22	25.35	45.49	18.95	14.13

Table 1 colorimetric measurement of *Bombyx mori* cocoons. Color components: L (Lightness), C (Chromaticity), h (hue angle, from arctangent b/a (0° = red purple, 90° = yellow, 180° = bluish-green, 270° = blue)) and Y (luminance), Z and X (spectral weighting curves) were measured and

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converted in the a* (bluish-green/red-purple hue component), b* (yellow/blue hue component) values (McGuire, 1992).

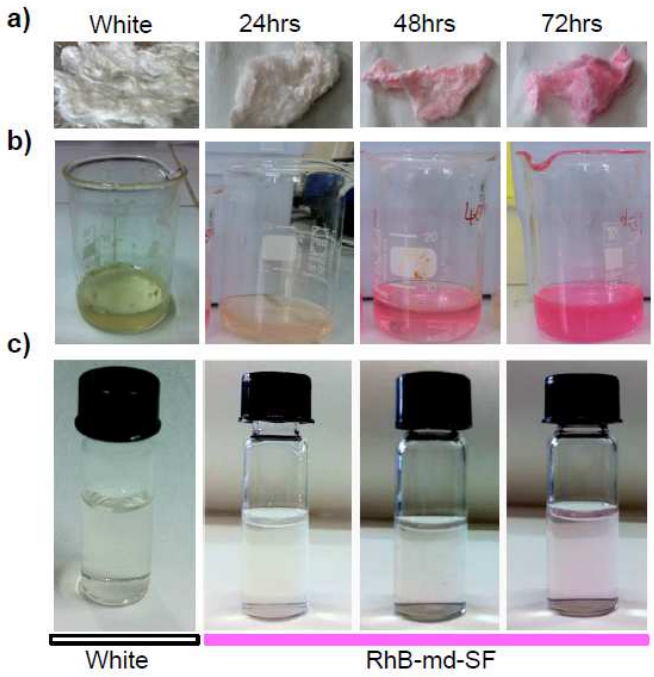

Figure 2. a) degummed silk fibres obtained from white and RhB-modified diet cocoons (reported in Figure 1 c-f). b) SF solubilized in LiBr solution. c) Regenerated SF solutions extracted from white and RhB-modified diet fibres.

We next separate the silk fibre by sericine, the glue like protein that couple silk wires together in the fibre, by degumming procedure (for details see Experimental section). Of note, the resulting RhB-md-SF fibres (Figure 2a right panels) were coloured with respect to those obtained from white cocoon (Figure 2a left panel) and displayed different shades of violet depending on the time of exposure of the worms to the RhB-modified diet. The same gradient difference in colour from white, 24h, 48h and 72h RhB-modified diet cocoon was retained when silk fibroin fibres were melted in LiBr (Figure 2b), and after the purification procedure (dialyses and centrifugation), (Figure 2c).

These data were in agreement with Tansil et al. [9] work as they showed that, eventhough a part of xenobiotics colorant is lost after degumming, degummed silk fibre retains doping agent[9].

Spectroscopic Properties of Regenerated SF Solution from Rhodamine doped diet feed cocoon.

The main aim of this work is to analyse chemo-physical properties of RSF and films obtained from 24h, 48h and 72h RhB-modified diet cocoon to explore the suitability of diet modified method for biomanufacturing of optically active silk technological substrates.

In this view, we next perform the UV-VIS optical properties of the obtained RSF (4% p/v) obtained from white SF, and from cocoon feed for 24h, 48h and 72h RhB-modified diet (RhB-md-SF).

The UV-VIS optical properties of SF are determined by the presence of two chromophore groups: the amino acid residues tyrosine (Tyr) and tryptophan (Trp) that display the maximum UV-VIS absorbance at the wavelengths 274 and 295 nm, respectively. Moreover, when the protein samples are excited at 274 nm and 295 nm, they emit fluorescence at 305 nm and 350 nm due to contributions of phenolic and indolic rings of Tyr and Trp, respectively.[11] The RhB is an extrinsic fluorophore, i.e. an organic molecule designed to identify and investigate biological structures and biochemical functions; Rhodamines absorbs in the 540-550 nm range depending on the type of Rhodamine dye (i.e RhB, Rh123, Rh101, Sulforhodamine) and emits fluorescences in the orange spectral region[12] (570-600 nm).

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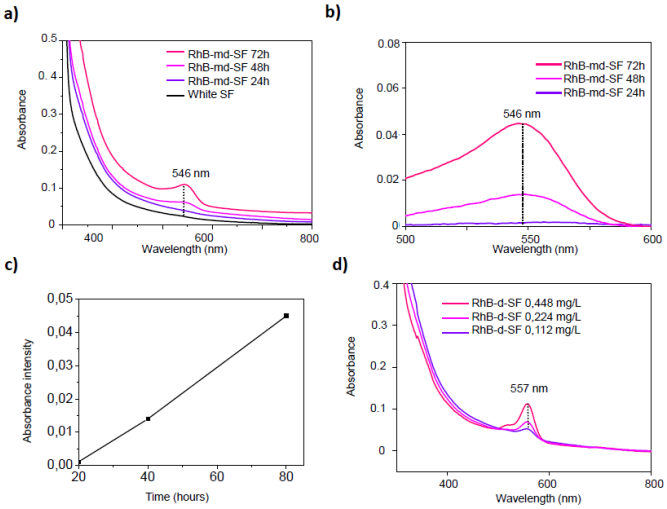

Figure 3. a-b) UV-VIS absorption spectra of regenerated SF water-solutions collected in the 300-800 and 450-650 nm regions. c) Maximal UV-VIS ABS values versus time (24, 48, 72 hours) of exposure of larvae to diet containing RhB. d) UV-VIS absorption spectra, in the 300-800 nm region of RhB-doped SF at different concentrations of dye (0,448 mg/L; 0,224 mg/L; 0,112 mg/L).

Figure 3a shows the UV-VIS absorption spectra of white and RhB-md-SF solutions, collected in the 300-800 nm; the white and as well RhB-md-SF fibroin solutions were not completely transparent and, hence, the UV-VIS spectra showed scattering-phenomena, possibly due to the presence into RSF of fibroin nano-micro aggregates.[13] Therefore, in order to emphasize the dye absorption peak in RhB-md-SF fibroin solutions in the 400 - 600 nm spectral range, the white SF solution was used as reference (fig. 3b). RhB-md-SF water-solutions displayed two main features in UV-VIS absorption spectra: a shoulder at 325 (Figure 3a) and a more pronounced peak at 546 nm (Figure 3a-b); for the white fibroin solution, just the absorbance at 325 nm appeared. The absorbance at 325 nm that appears in the tail of the typical protein maximum absorption range (274-295 nm) could be attributed to a small percentage of β-sheets structures[13], due to the presence of hydrophobic motifs (GAGAGS and GA(V)GAGY).

The absorbance at 546 nm, associated to chromophore group of RhB[12], increased gradually from RhB-md-SF24h to RhB-md-SF72h. In particular, the intensity values were almost proportional to the feeding time (24hs>,48> 72 hours) (Figure 3c).

When RhB was blended with SF and with water displayed the maximum absorbance at 557 (Figure 3d) and at 554 nm (see Figure S1 of Supplementary Information), respectively.

Therefore, the results of UV-VIS absorption of the RhB-md-SF fibroin solutions displayed the features of RhB. However, we observed that RhB has a slightly different optical behaviour depending upon the solvent.

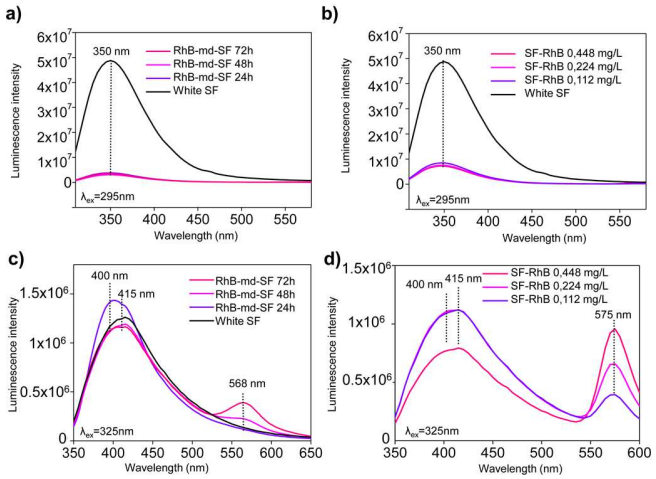

Figure 4. Emission profiles of white, natural coloured regenerated SF water-solutions and RhB-doped SF solutions recorded by exciting at a-b) 295 nm and c-d) 325 nm.

RhB-md-SF and RhB-d-SF water-solutions were excited at 295 nm (Figure 4a-b) and 325 nm (Figure 4c-d) to evaluate the behaviour of the amino acid residues. Upon excitation at 295 nm (Figure 4a-b), all fluorescence spectra displayed a wide band at 350 nm attributed to emission of Trp residues that are much more exposed to bulk water.[14]

When RhB-md-SF silk solutions were excited at 325 nm (Figure 4c-d), fluorescence spectra were characterized mainly by two features: a broad structured emission

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with maxima at 400 and 415 nm, and a peak at 568 nm, of increasing intensity from RhB-md-SF-48h to RhB-md-SF-72h. The latter peak was almost undetectable in the RhB-md-SF-24h. When excited at 325 nm, white SF fluorescence spectrum displayed only the twin bands at 400 and 415 nm (Figure 4c).

The emission profiles of RhB-d-SF samples, recorded exciting at the same wavelength (325 nm), , were comparable to those of natural coloured samples; indeed, the broad band (400 nm-415 nm) and the emission of dye are still present, although the latter was shifted from 568 nm to 575 nm (Figure 4d).

The emission at 400 nm is typical of the singly ionized dityrosine chromophore, in which one of the two phenolic hydroxyl groups is dissociated[15]. Another possible contributor to emission at 400 nm could be oxidized tryptophan[13]. However, the presence of these species (dityrosine and oxidized thryptophan) into regenerated SF water-solutions could derive from the treatments to which the protein chains are subjected during the various phases of extraction and purification of fibroin from cocoons. Some authors attributed the emission at 415 nm, visible in all silk solutions and resulted by exciting at 325 nm, to copper proteins containing type-3 centres[16]. In this work we have not investigated in detail the presence or less of copper ions in SF samples, but different researchers found that the copper and other inorganic ions (Fe, Na, Ca, K, Zn, Mn) are in the gland of silkworm playing an important role into folding process of the fibroin during the natural spinning-process[17]. Therefore, in our case we could hypothesize the presence of a low concentration of copper (II) ions complexed to specific peptide sequences into fibroin: AHGGYSGY, where the formation of complex is mainly due to the presence of imidazolic ring of histidine residues.

In order to examine the fluorescence features of RhB in the different systems, we excited the various solutions of RhB at 546 nm. In all cases the spectra were recorded from 550 nm to 750 nm.

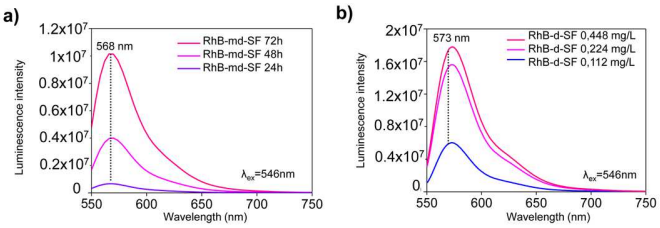

Figure 5. a-b) Emission profiles recorded by exciting at 546 nm natural coloured regenerated SF water-solutions and RhB-doped SF solutions.

When the RhB-md-SF solutions were excited at 546 nm (Figure 5a), the fluorescence spectra displayed the maximum intensity at 568 nm. As well as the UV-VIS absorption also the intensity of emission increased from RhB-dm-SF 24h to RhB-dm-SF 72h. As expected, no emission was observed when exiting SF water-solution at 546 nm. On other hand, RhB-doped SF (Figure 5b) and RhB-w (S2 of Supplemetary Information) solutions displayed almost the same maximum emission at 573 and 575 nm, respectively. Fluorescence bands of RhB-d-SF solutions were of greater intensity than those of RhB-md-SF samples.

Spectroscopic Properties of Regenerated SF Film from Rhodamine doped diet feed cocoon.

We next investigated the chemophysical and conformational properties of SF films realized by drop-casting and slow-drying approach, by means of optical transmittance and fluorescence spectroscopy to verify their suitability to be used as substrates in optical and photonic devices. The SF films made by white and RhB-md-SF24h solutions were completely optical transparent, (up to 95%) in the visible region (300-800 nm) with a clear consistent decrease under 277 nm as a result of protein absorbance. On the other hand, the RhB-md-SF 48h and RhB-md-SF-72h films showed a

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small loss in transparency around 550 nm, due to RhB absorption (Figure 6b).

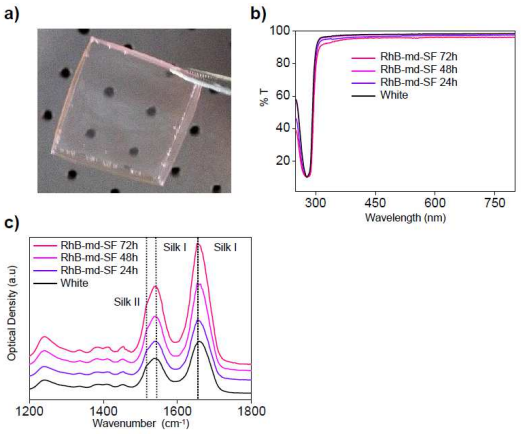

Figure 6. a) Natural coloured fibroin film obtained from RhB-md-SF 72h solution and prepared by drop-casting and slow-drying method. b) Trasmittance properties of white and natural coloured regenerated SF films recorded from 250 to 800 nm. c) IR-FT spectra of white and natural coloured regenerated SF films collected in the amide regions (1200-1800 cm^{-1}).

Vibration modes are used in order to investigate the secondary structures of SF protein processed in a specific form. In particular, FT-IR analysis in the spectral regions of the bands of amide I (1700-1600 cm^{-1}), amide II (1600-1500 cm^{-1}) and amide III (1350-1200 cm^{-1}), allows to distinguish among different silk conformations: water-soluble silk I and water-insoluble silk II.[18] The vibration structures of the amide I region are due to: C=O stretching (80% of contributions), NH in-plane bending, the out-of-phase CN stretching and the CCN deformation. The absorptions of the amide II region are, mainly, a result of the CN stretching and the NH in-plane bending vibrations of the polypeptide backbone.[18] In figure 6c the IR spectra of silk films obtained from white and RhB-md samples, which were fabricated by drop casting and slow drying approach, are reported.

The IR spectra of all RhB-md samples do not show any relevant difference compared to white SF films (Figure 6c) indicating that the presence of RhB does not influence the self-assembling of fibroin chains during the film formation. The vibration peaks recorded for all SF samples were: 1239 cm^{-1} in the amide III region, 1515 and 1539 cm^{-1} in the amide II, 1658 cm^{-1} in the amide I spectral range. Except the value to 1515 cm^{-1}, typical of silk II and assigned to vibration mode of the Tyr (CC stretching of the aromatic ring and CH bending of the side chain),[18b] the others absorptions indicated the prevalence of water-soluble SF conformation (silk I). This result was in agreement with the data previously described for films prepared by the same slow drying method,[3] and confirms that silk fibroin film self-assembly properties were not modified by doping diet method.

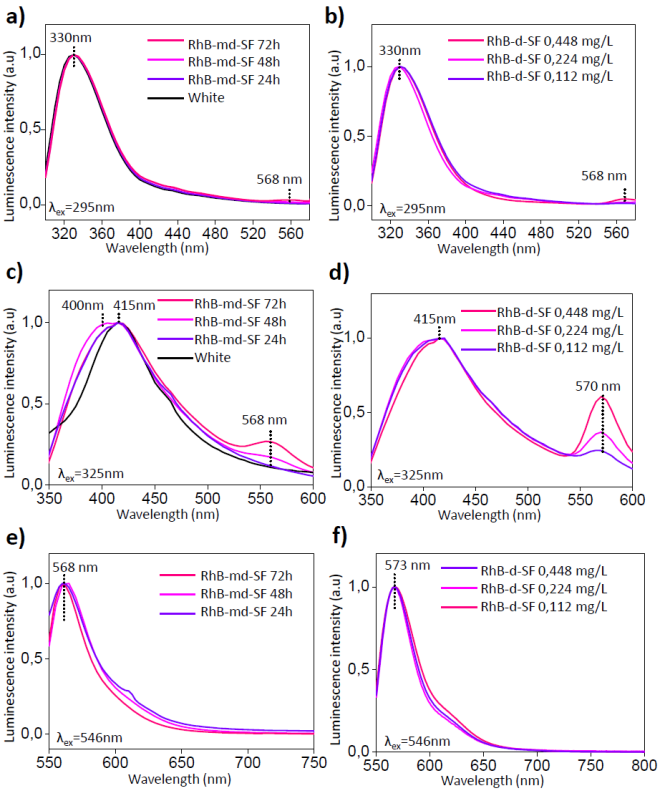

Figure 7. Emission profiles of fibroin films obtained from white, natural coloured SF and RhB-doped SF solutions. The fluorescence spectra were recorded by exciting at: a-b) 295 nm, c-d) 325 nm, e) 546 nm and f) 557 nm. Fibroin films were prepared by drop-casting and slow-dying approach on quartz substrates.

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The same paradigm applied to SF solutions was used for all SF films in order to collect fluorescence profiles. By exciting at 325 nm and at 546 nm, we did not observe any difference in the spectral features of RhB-md solutions (Figures 5 and Figure 6) and films (Figure 7c and 7e). When exciting at 295 nm we observed the emission of Trp residues at 330 nm, that some authors assigned to Trp residues enable to give H-bonded exciplexes[14b] (Figure 7a). The formation of H-bonded exciplexes could be a result of loss of water during the formation of films. Hence the Trp, into polypeptide chains could not behave like amino acidic free in the bulk water.

The fluorescence spectra of RhB-d-SF films were collected also by exciting at 295 nm, 325 nm and 546 nm (Figure 7b, 7d and 7f, respectively). The samples displayed protein luminescence features more comparable to those of RhB-md-SF films, in particular for the excitation at 295 nm where the peak at 330 nm appeared (Figure 7b). On other hand, the signal of dye was different for the emission wavelength (573 nm instead of 568 nm) and form of the curve (Figure 7f). This could be associated to a different assembling or bonding of molecules when the RhB is dispersed in the white SF solutions.

Conclusions

The main finding of this work is that diet addition method is suitable for biomanufaturing of doped and optically active silk solution and film. The RhB-md-SF films were optically transparent and fluorescent with UV-Vis properties typical of RhodamineB added to white natural regenerated silk fibroin solution. The present work open the view for the biomanufacturing of different silk based technological substrates that take advantage of producing innovative functionalized and technological material by structural production, self-assembling and modification by living organisms, without the need of time-consuming, expensive, post-processing chemical modification using organic solvent. Because of large-scale cultivation of silkworms for the textile industry, there are abundant and reasonable cost sources for this natural polymer. Indeed, it has been envisaged that coloured and luminescent silk fibres offer several advantages for the textile industry. Applicability of the same methods for the use of silk as technological substrates for biophotonics optics and optoelectronic could encourage application of greener methods for global and sustainable manufacturing.

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Notes and references

[a] Laboratorio di Micro e Submicro Tecnologie abilitanti dell'Emilia-Romagna (MIST ER), Via P. Gobetti 101, I-40129 Bologna, Italy

[b] Consiglio Nazionale delle Ricerche- Istituto di Biometeorologia (CNR-IBIMET), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: n.divirgilio@ibimet.cnr.it; Tel: +39-051-6399004

[c] Consiglio Nazionale delle Ricerche-Istituto per lo Studio dei Materiali Nanostrutturati (CNR-ISMN), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: g.ruani@bo.ismn.cnr.it; Tel: +39-051-6398508

[d] Consiglio Nazionale delle Ricerche – Istituto per la Sintesi Organica e la Fotoreattività (CNR-ISOF), via P. Gobetti 101, 40129 Bologna, Italy. E-mail: Valentina.benfenati@isof.cnr.it; Tel: +39-051-6399760

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† These authors equally contributed to this work.

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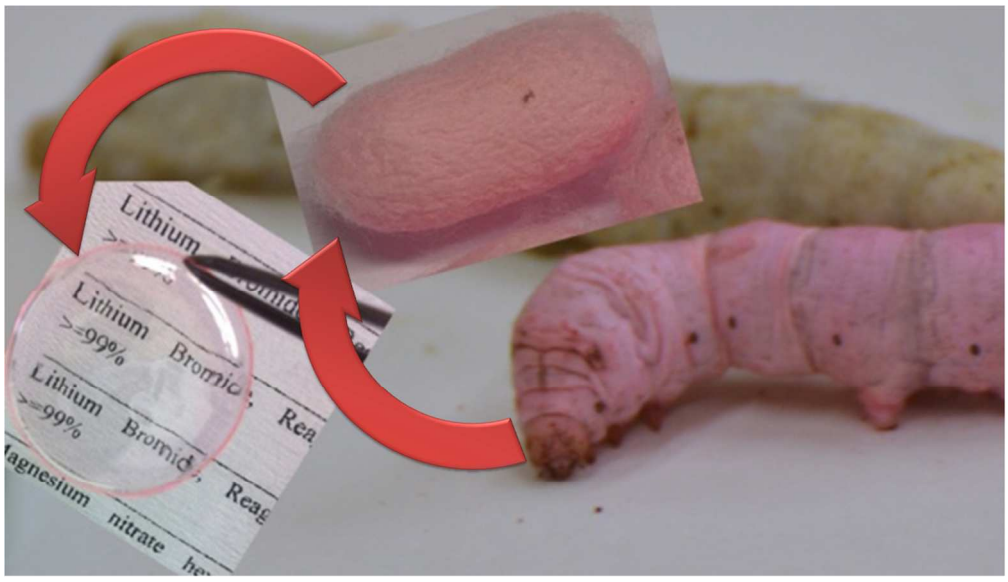

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