Investigating silk yield and morphological changes in silk fibres obtained from silkworms fed with Ag and/or TiO₂ nanoparticles

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Abstract. Natural polymers like silk can be augmented *in vivo* with metallic elements to improve their intrinsic properties and stability. This study demonstrates the uptake of Ag and/or TiO₂ nanoparticles as diet supplements by silkworms. Nanoparticles dose on morphological changes in silk fibroin fibres and a change in overall silk yield was investigated. Though significant fibre reinforcement with Ag and/or TiO₂ was not noticed; an increase of 6% in the silk production was observed when silkworms fed with a particular composition of Ag + TiO₂ nanoparticles (0.37 mM each) in their diet. Here, an average diameter of the degummed silk fibre was $\sim 10.91 \mu\text{m}$ with a roughness parameter value R_q being 134. This value is comparable to silk fibres obtained from silkworms fed with Ag + TiO₂ (0.22 + 0.53 mM) nanoparticles and this R_q value was also low when compared to the other tested compositions with nanoparticles. Silks from the control samples (devoid of nanoparticles in their feed) had high diameter fibres and are with low R_q values and silk yield. However, nanoparticle supplementation to the silkworm's diet resulted in low diameter silk fibres with varying roughness and with improved silk yield for the tested doses.

Keywords. Silk yield; diet supplements; fibre reinforcement; nanoparticles.

1. Introduction

Silk fibres have a significant use in the textile industry and biomedical field for many years owing to their biocompatibility and controllable biodegradability and they show excellent mechanical properties [1,2]. These fibres are not stronger when compared with their rival spider silk, but have certain advantages over them as they are domesticated easily and can produce silk in large amounts [1,3]. Modification of silkworms and silk fibres is an active research area where *in vivo* and *in vitro* transformations are explored to improve the silk yield, fibre properties and resistance towards viral diseases [1,4–8]. For instance, modification of silk fibres with nanoparticles like Ag, TiO₂ and TiO₂@Ag are examples of *in vitro* studies and changing the diet of silkworms with the addition of dyes and metallic elements like TiO₂ are some examples of *in vivo* studies [1,2,5,6,9]. Among the stated methods, feeding method modification is promising as it limits the wastage of materials like nanopowders, dyes and other additives [1]. Materials as feed additives can easily be integrated with natural silks during silk production by silkworms and can give high-performance functional silks on a large scale [1,4].

Nanoparticles like Ag and TiO₂ were proved to be efficient with synthetic and natural fibres with new functionalities

and uses [10–13]. Regarding healthcare applications, usually, natural fibres are of the first choice over synthetic fibres as they are not harsh on the skin and can be produced at low cost [14,15]. Aiming at natural fibre modification, in this work, nanoparticles of Ag and/or TiO₂ are fed to silkworms as diet supplements and the silk fibres obtained from them are explored for unusual behaviour if any and also observed for nanoparticle assimilation with fibres. With different doses of nanoparticles; important factors like the yield of silk, average diameter and roughness parameter of silk fibres produced are also investigated. Unlike the above ascribed reported studies, the outcome of this study helps to identify the effective dose of nanoparticles (Ag and/or TiO₂) required to improve silk yield as well as to have fibres with UV protection (due to TiO₂) and biostability (due to Ag).

2. Materials and methods

2.1 Silkworm rearing and nanoparticle feeding

The first day of 5th instar *Bombyx mori* silkworms were provided by Silkworm Culture and Reeling Unit, Chebrolu, Andhra Pradesh. The larvae were raised in trays (of size

Table 1. Different samples for diet modification of silkworms.

Sample	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Ag + TiO ₂ , mM	0 + 0.72	0.22 + 0.53	0.37 + 0.37	0.53 + 0.22	0.72 + 0

60 × 30 × 6 cm) with breathable mesh design. Silkworms in control and test experiments were fed with normal mulberry leaves until the first day of 5th instar. For experimental groups, mulberry leaves were sprayed with 20 ml of Ag and/or TiO₂ nanoparticles as detailed in table 1. These leaves were dried naturally at room temperature and used to feed the 5th instar larvae from its second day in its third feeding every day until their spinning.

2.2 Preparation of nanoparticles

Aegle marmelos leaf extract (LE) was prepared as described in our previous study [16] for the synthesis of Ag nanoparticles of ~60 nm. During the synthesis, 1.0/0.3 proportion of AgNO₃ (mM)/LE (%) was maintained and Ag nanoparticles were prepared by the reduction of 8 mM AgNO₃ by the LE. Nano-TiO₂ (~25 nm) was from Sigma Aldrich, India and respective stock solutions were prepared according to the need.

2.3 Degumming step and silk percentage calculation

In a degumming step, primarily, cocoons (control and tests) were subjected to drying steps as stated in [1], for 1 h at 110°C and then, for 3 h at 80°C in a vacuum drying oven. The obtained cocoons without insects were peeled into pieces and treated three times with 0.5 wt% aqueous Na₂CO₃ solution at 100°C for 30 min. Now, the samples obtained were washed with deionized water and naturally dried to obtain degummed silks.

Consecutively, the silk percentage is estimated as follows; cocoons obtained from control and experimental groups are weighed separately and the percentage of the cocoon shell is only assessed. This estimate is the direct measure of raw silk content and raw silk yield in percentage can be known by the following method:

$$\text{Silk yield} = \frac{\text{weight of cocoon shell (g)}}{\text{weight of whole cocoon (g)}} \times 100.$$

3. Results and discussion

The effect of nanoparticles as a feed supplement on growth and silk yield from silkworms is stated as follows; figure 1 shows the photographs of the silkworms (control and test samples), cocoons and scanning electron microscopy (SEM, JEOL-2100F) images of the degummed silk fibres upon

diet modifications, respectively. As shown in figure 1, the silkworms (control/test samples) and their cocoons were observed to be in the same colour, size and configuration with no significant physical appearances. However, upon close magnification in SEM (figure 1), it is observed that there are differences in surface smoothness of degummed cocoon fibres of each sample. From the SEM images (figure 1), it can be seen that nanoparticles are not evident on the surface of the silk fibres. Furthermore, it is also observed that nanoparticles addition to the diet of silkworms did not cause any reduction in food intake or death. As of microscopic observations, the diameters of the degummed fibres from various samples fed with nanoparticles, their silk % and the average roughness parameter values in terms of root mean square deviation (R_q) are listed in table 2. The surface roughness parameter was determined by the approach proposed by Ondimu and Murase [17] and all the calculations were performed using Image J analysis software. It is observed that the diameters of fibres from the control sample are higher than the other tested samples fed with nanoparticles. As all other feeding conditions are the same in control and test samples, this behaviour might be merely due to nanoparticle supplementation to the silkworm's diet. Our observations were supported by a reported study where the authors used TiO₂ nanoparticles alone and observed a reduction in the average diameter in degummed silk fibres [1]. Here, they mentioned confined crystallization of silk fibroin which will restrict the movement of protein molecules and decrease the nucleation sites of crystallization in silks with increasing TiO₂ as a feed additive.

Table 2 states that the silk % was more in the sample of Ag + TiO₂ (0.37 + 0.37 mM) where the average diameter is smaller than the other studied samples. The surface roughness of this sample is also low and near to the sample Ag + TiO₂ (0.22 + 0.53 mM) when compared to other tested samples. The nanoparticle supplementation, especially individual Ag and TiO₂ to the diet of silkworms were studied by various research groups and growth stimulant activity was observed [4,9]. These studies postulated that the addition of nanoparticles could improve both protein and carbohydrate metabolisms along with an increase of silk yield. TiO₂ supplementation alone can improve the absorption and utilization of amino acids from the feed [9] and their 1% addition to the diet of silkworms could improve breaking strength and UV resistant property of silk [1]. Complementing the above reports, our study showed an increase in the silk yield with a relatively low dose of Ag + TiO₂ nanoparticles to silkworm's diet.

Figure 1. Effect of Ag and/or TiO₂ content in the diet on growth, cocoons and degummed silk fibres obtained from silkworms after the 5th instar. Representation is from left to right in the figure for each sample and the scale bars in the SEM images of degummed silk is 2 μ m (in low magnifications) and 1 μ m (in high magnifications).

Table 2. Average diameter, silk % and roughness parameters of the degummed silks from various samples.

Sample, mM	Average diameter, μm	Silk, %	Roughness parameter, R_q
Control	12.406	20.1	102.78
Ag + TiO ₂ (0 + 0.72)	12.069	20.3	178.333
Ag + TiO ₂ (0.22 + 0.53)	11.734	22.75	132.714
Ag + TiO ₂ (0.37 + 0.37)	10.919	26.08	134.008
Ag + TiO ₂ (0.53 + 0.22)	12.341	19.51	159.459
Ag + TiO ₂ (0.72 + 0)	12.047	20.41	154.604

Figure 2. FTIR spectra of degummed silks.

In addition to the above, the Fourier-transform infrared spectroscopy (FTIR, Thermo Fisher Scientific, Nicolet iS10) spectra of degummed natural silk fibres compared with silk fibres obtained from diet supplementation with nanoparticles (Ag and/or TiO₂) are shown in figure 2. Spectra from the figure show characteristic peaks at 3390, 2880, 2780, 1650, 1460, 1170, and 1055 cm⁻¹, respectively. The peak at ~3390 cm⁻¹ is due to -NH stretch of the amide group and the peaks at ~2880 and 2780 cm⁻¹ arise from alkane -CH groups in silk proteins. The peaks at ~1650 and ~1170 cm⁻¹ may be attributed to the carbonyl (C=O) stretching vibration and phenolic OH groups of Tyr, the peak at 1460 cm⁻¹ may be assigned to carboxylate groups and finally, the absorption peak at ~1055 may be due to (-C-O-) groups in silk proteins. The peak positions observed are similar in findings with other published reports [18–20] and the identical peak positions of all the tested samples in figure 2 indicate that the nanoparticle supplementation did not change the chemical nature of silk fibres.

Even though FTIR results indicate no significant differences, there are noticeable changes in the structural diameter and roughness parameter between the control and modified-silks as detailed in table 2. Upon comparison, the

modified silks exhibited low-average diameters and higher roughness values than the control silks. To make sure that the stated differences are due to nanoparticles in silkworm diet supplementation; energy-dispersive X-ray (EDX) analysis was performed on the selected fibre samples after degumming (figure 3) and the contents of Ag and Ti were observed. The wt% of Ag and Ti are found to be low in the tested samples and are correlating with the increasing order of dose fed to silkworms in their diet. From figure 3, one can see an increase in Ag content when silkworms are fed with more Ag in their diet and vice versa for TiO₂ also. But as EDX analysis only considers the superficial layers of the coated samples in elemental estimation and may be inaccurate in exact quantitative estimation from the overall sample. So, for accuracy, the content of Ag and Ti of the selected samples are quantified using inductively coupled plasma mass spectrometry (ICP-MS) analyses as listed in table 3. It is clear from table 3 that the addition of nanoparticles increased the content of Ag and Ti in silk fibroin fibre. Moreover, the rate of increase in their amount varied for individual tested samples of the same concentration. Therefore, it can be concluded that there is a significant variation in the uptake of Ag and Ti when silkworms are fed with similar concentrations of Ag and/or TiO₂ nanoparticles. It is certain that silkworms accept and excrete nanoparticles if given as feed supplement [6,9,21] and this amount may vary with the type of nanoparticles as detailed in our case.

When compared to the reported studies [1,4,6,9,21] involving nanoparticle supplementations to their diet, our study is different from its kind where mixtures of nanoparticles (Ag and/or TiO₂) in low doses were given as dietary supplements to silkworms. The results of our study are encouraging as a recent study by Meng *et al* [21] pointed out that low-doses of nanoparticles in feeding will help in growth and cocoon weights of silkworms effectively. This study with silkworms and nanoparticles attained cocoons with more silk % and silk fibres with reasonably high average diameter. Our approach of a feeding method can help in the generation of silk fibres with improved functional properties and has huge scope to be applied on a large scale in silkworm rearing units.

Figure 3. EDX spectra of the degummed silks obtained from diet supplementation of silkworms with various doses of Ag and TiO₂ nanoparticles. Scale bars in the SEM images is 20 μ m.

Table 3. The content of Ag and Ti in degummed silk fibres from 0.1 g sample.

Silks from Ag + TiO ₂ treatment (mM)	Content (ppm)	
	Ag	Ti
0 + 0.72	—	6.59
0.22 + 0.53	1.36	6.45
0.37 + 0.37	1.39	5.47
0.53 + 0.22	1.44	5.26
0.72 + 0	1.48	—

4. Conclusion

In this study, silk fibres were collected and studied after raising the silkworms with different doses of Ag and/or TiO₂ nanoparticles as diet supplements. Compared with those of the control silk fibres; with Ag + TiO₂ (0.37 mM each) in their diet, silk yield and roughness parameters were increased by 6 and 1.3%. In this case, a 12% decrease in average diameter was also observed. But upon functional group analysis, all the tested samples with nanoparticles inferred that the silkworm fibres are structurally similar to no vital differences. Though nanoparticle evidence is absent in their fibre structures; ICP-MS results detected that there is Ag and/or TiO₂ in its structure and the quantities were dose-dependent. Thus, functional silk fibres with protective properties were produced in a facile way by simple diet modification of silkworms. Furthermore, this study displayed that enhanced silk yield could be possible when silkworms fed with nanoparticles (Ag and/or TiO₂).

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References

- [1] Cai L, Shao H, Hu X and Zhang Y 2015 *ACS Sustain. Chem. Eng.* **3** 2551
- [2] Fei X, Jia M, Du X, Yang Y, Zhang R, Shao Z *et al* 2013 *Biomacromolecules* **14** 4483
- [3] Andersson M, Johansson J and Rising A 2016 *Int. J. Mol. Sci.* **17** 1290
- [4] Mathivanan V, Ganesh Prabu P, Selvisabhanayakam B D, Pradhapan M and Vivekananthan T 2012 *Int. J. Res. Biol. Sci.* **2** 60
- [5] Tang B, Li J, Hou X, Afrin T, Sun L and Wang X 2013 *Ind. Eng. Chem. Res.* **52** 4556
- [6] Cheng L, Huang H, Chen S, Wang W, Dai F and Zhao H 2017 *Mater. Des.* **129** 125
- [7] Li B, Xie Y, Cheng Z, Cheng J, Hu R, Gui S *et al* 2012 *Biol. Trace Elem. Res.* **150** 221
- [8] Sheikh F A, Ju H W, Moon B M, Park H J, Kim J H, Lee O J *et al* 2013 *Nanoscale Res. Lett.* **8** 303
- [9] Ni M, Li F, Tian J, Hu J, Zhang H, Xu K *et al* 2015 *Biol. Trace Elem. Res.* **166** 225
- [10] Perelshtein I, Applerot G, Perkash N, Guibert G, Mikhailov S and Gedanken A 2008 *Nanotechnology* **19** 245705
- [11] Dong P, Cheng X, Huang Z, Chen Y, Zhang Y, Nie X *et al* 2018 *Mater. Res. Bull.* **97** 89
- [12] Khude P, Majumdar A and Butola B S 2018 *Fibers Polym.* **19** 1403
- [13] Qin Y 2019 in Rajendran S (ed.) *Advanced textiles for wound care*, 2nd edn, The Textile Institute Book Series (UK: Woodhead Publishing) p 193
- [14] Haug S, Roll A, Schmid-Grendelmeier P, Johansen P, Wüthrich B, Kündig T M *et al* 2006 *Biofunct. Text. Skin* **33** 144
- [15] Mason R 2008 *J. Fam. Health Care* **18** 63
- [16] Rao K J and Paria S 2013 *Mater. Res. Bull.* **48** 628
- [17] Ondimu S and Murase H 2008 *IFAC Proc.* **41** 641
- [18] Dong Q, Su H and Zhang D 2005 *J. Phys. Chem. B* **109** 17429
- [19] Chen X and Wei L 2014 *J. Eng. Fibers Fabr.* **9** 6
- [20] Khanchaiyapoom K and Prachayawarakorn J 2008 *J. Met. Mater. Miner.* **18** 237
- [21] Meng X, Abdlli N, Wang N, Lü P, Nie Z, Dong X *et al* 2017, *Biol. Trace Elem. Res.* **180** 327