AFM STUDY OF EGG SAC SILK FIBERS OF THE GIANT WOOD SPIDER, *NEPHILA PILIPES* (ARANEAE, ARANEIDAE)

Sawarkar A. S.[a] and Sawarkar S. B.[b]

[a] Department of Zoology, Shri. R. L. T. College of Science, Akola 444001, India

[b] Department of Physics, Shri. Shivaji College of Arts, Commerce & Science, Akola 444001, India

A B S T R A C T

Silk production from the abdominal silk glands is unique to spiders which have made them successful in any habitat on the earth surface. Various kinds of silk producing glands in spider function as small biofactories. The egg sac silk is produced from the cylindrical glands by female *Nephila*, immediately after egg laying. In the present work, atomic force microscopy was used as a qualitative measurement tool for determining the microscale topography and a quantitative tool for the surface nanostructure of egg sac silk fibers of *Nephila pilipes*. Two dimensional deflection images of egg sac silk fibers were recorded in contact mode. Image analysis was performed with WSxM Nanoscope image processing software. The topography of egg sac silk fibers is not uniform. Patches of this silk thread showed alternate smoother and rougher surface. Such structure increased the surface roughness of these fibers and might have assisted the spiderlings in attaching themselves. Roughness analysis of egg sac silk suggested that, this biomaterial has high toughness that may be suitable for dissipating high amounts of mechanical energy. Also, nanocrystalline and amorphous regions in spider silk fibers enrich them with strength and elasticity. This study of structure–function relationships can guide the production of high quality and smart bio-material with recombinant DNA technologies.

Keywords: Nephila pilipes, Egg sac silk, AFM

1. Introduction

All spiders produce protein-based biopolymer in the form of silk. Spider silk is known for its outstanding properties. Due to its desirable properties, spider silk is studied intensively to better understand the interplay between its structure and performance. Li et al. (1994) used atomic force microscopy to study the three-dimensional nanometer scale structure of dragline silk of Nephila clavipes from microtomed sections of the silk. Trancik et al. (2006) used Transmission Electron Microscopy and x- ray diffraction to examine the nanostructure of spider dragline silk from cob web weaving spider, Latrodectus hesperus. Benmore et al. (2012) performed total x-ray scattering experiments of dragline silk from Nephila clavipes, Argiope aurantia and Latrodectus hesperus. Jeffery et al. (2018) overviewed experimental and computational studies that have provided a wealth of detail at the molecular level on the highly conserved repetitive core and terminal regions of spider dragline silk. Most of the studies are focused on study of dragline silk.

Immediately after egg laying, egg sac is constructed by female spider. All spiders package their eggs in silken egg sacs, or cocoons to protect them against physical and biotic threats, create a suitable microclimate both for embryonic development and hatching, and protect early postembryonic stages until they leave the cocoons (Gheysens et al.,2005). In order to understand what makes egg sac silks mechanically superior, it is necessary to understand its structural organization and properties. AFM is a nondestructive technique which can provide rich topographic images of the silk fiber. The micrometer and nanometer scales affect different aspects of cell behavior and different cell type react differently to different surface topography (Zinger et al., 2004); hence, it is important to study the surface roughness over the range of length scales.

2. Experimental

For egg sac silk, gravid female was collected and placed in the ventilated plastic container. It was fed with a cockroach after every two days. After some days, she laid eggs and immediately started to spoon egg sac silk to cover it. The silk fibers from the middle portion of the egg sac were used for AFM study. Silk threads were fixed on the thin glass slide with the help of adhesive transparent tape at both ends. Then it was mounted on piston with the help of double sided sticking tape. Piston was fixed on sample holder. Atomic force microscope, Nanoscope E, model no. 245 was used for imaging silk samples. Two dimensional deflection images of egg sac silk were recorded in contact mode. Image analysis was performed with WSxM Nanoscope image processing software.

3. Results and Discussion

Atomic force microscopy (AFM) was used to obtain topographic images of silk sample and to measure surface roughness of spider egg sac silk. Fig. - 1 and 2 shows representative surface topographies from AFM images of Nephila pilipes silk samples. The arrow direction indicates the fiber axis. These images were also used for calculation of surface roughness values of silk. Fibers of the egg sac silk did not exhibit the uniform surface features. Patches of this silk thread showed alternate smoother (Fig.-1, a to d) and rougher surface ((Fig.-2, a to d). AFM images showed superimposed nanostructures on silk surface having darker and lighter color. In AFM, dark areas depict low features and white areas depict high features. Therefore, the white portion from the figure is the projection from base matrix (Rajeev et al., 2001). The peculiar topographical structure of spider egg sac silk may be due to presence of secondary structural elements like β-sheets, β-turns, α-helices etc. These structures give semicrystalline nature to spider silk

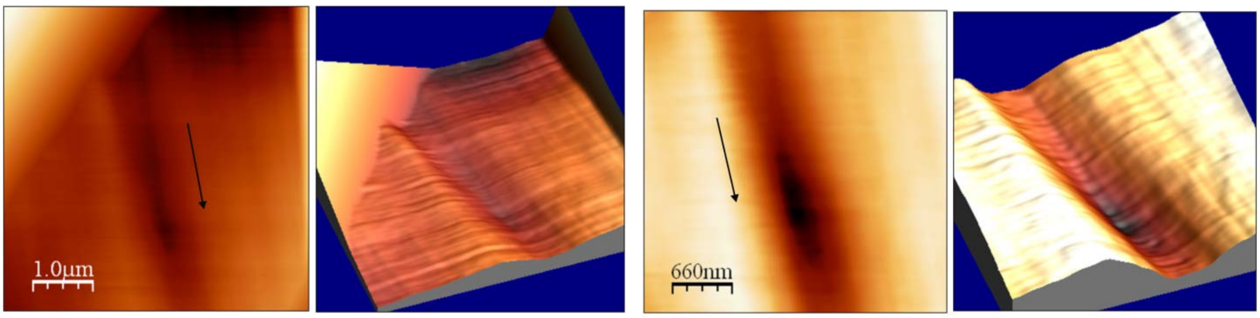

Fig. 1- AFM images showing topography of smoother region of egg sac silk of *Nephila pilipes*
(a) 2D image scan size 5 x 5 µm^2 (b) 3D image scan size 5 x 5 µm^2
(c) 2D image scan size 3.3 x 3.3 µm^2 (d) 3D image scan size 3.3 x 3.3 µm^2

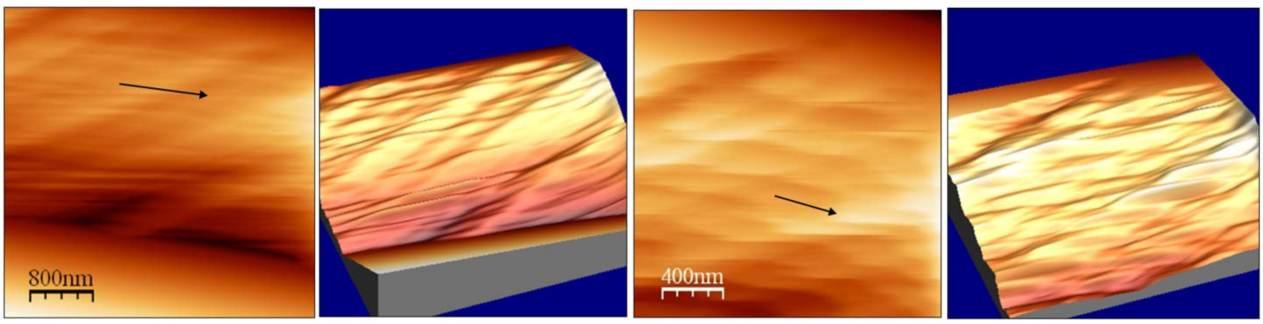

Fig. 2- AFM images showing topography of rougher region of egg sac silk of *Nephila pilipes*
(a) 2D image scan size 4 x 4 µm^2 (b) 3D image scan size 4 x 4 µm^2
(c) 2D image scan size 2 x 2 µm^2 (d) 3D image scan size 2 x 2 µm^2

For roughness analysis, three parameters were measured: the root mean square (RMS), the arithmetic average height (Ra) and the maximum height of hills (H). RMS represents the standard deviation of the height values within the given area and allows the surface roughness to be determined by statistical methods. Ra is the most frequently used roughness parameter. Roughness values changed with the scan size (Table - 1). Hence, the measurements were performed using different scan windows. The characterization of material surface roughness on different length scale is important because biocompatibility of material is dependent on material chemistry and physical features as well as on surface roughness (Huang *et al.*, 2004).

Table 1 - Surface roughness: RMS, Ra and H for egg sac silk of *Nephila pilipes*

Scan Size	R.M.S.(nm)	Ra(nm)	H(nm)
5 x 5 μm^2	295.14	505.83	1773.18
3.3 x 3.3 μm^2	116.58	306.26	507.1
4 x 4 μm^2	323.72	717.02	1770.79
2 x 2 μm^2	254.87	797.38	555.43

The silk fibers of egg sac possess particular mechanical properties that allow the creation of a particular three dimensional structure. Egg sac silk creates a protective layer against the trauma, as well as against predators and parasites (Guarisco, 2001), providing an approximate microclimate mediating against temperature and humidity fluctuations for embryonic development, hatching and subsequent molting and durable shelter for spiderlings (Austin, 1985). Females of *Nephila pilipes* lays eggs in the month of November-December and young ones of spiders get hatched at the beginning of July. These eggs should be protected in good condition for more than six months. Cushion of egg sac silk provides protection to the future generation of spider. X- ray diffraction pattern of egg sac silk of *Nephila pilipes* confirms its semicrystalline nature. These nanostructural features of spider silk plays very important role in enhancing mechanical performance of silk threads (Amaley, *et al*., 2014). The effect might be even more strongly selected for egg sac silk to protect developing eggs, which might be particularly adopted to resist decomposition or destruction by predators, parasites or fluctuations in external abiotic conditions (Zhao *et al*., 2005).

4. Conclusion

Nanostructural features of egg sac spider silk plays very important role in enhancing mechanical performance of silk thread. Surface roughness values of this silk indicate that, it is designed for extreme toughness. Hence, it can be concluded that, spider silk having high and stable mechanical performance is found in nature. Successful large scale production of this beautiful, naturally pink colored spider silk with DNA recombinant technology will definitely open a new gate in textile sector.

Acknowledgement

We are thankful to UGC-DAE Consortium of Scientific Research, Indore (Madhya Pradesh, India) for providing AFM facility.

References

1. Amaley, A.H., Sawarkar, S.B., Tamgadge, Y.S. & Palhade, S.M. (2014). X- ray diffraction study of egg sac silk of *Nephila pilipes* (Araneae: Nephilidae). *Proceedings of NCRTMPA-2014, S.K. College, Akola (MS),* 213-215.
2. Austin, A.D. (1985). The function of spider egg sac in relation to parasitoids and predators, with special reference to Australian fauna. *J. Nat. His.* 19, 359-376.
3. Benmore, C.J., Izdebski, T. & Yarger, J.L. (2012). Total x-ray scattering of spider dragline silk. *Phys. Rev. Lett*., 108(17), 178102.
4. Jeffery L.Y., Brian R.C. & Arjan v.d.V. (2018). Uncovering the structure–function relationship in spider silk. *Nature Reviews Materials,* 3, Article no: 18008, DOI- https://doi.org/10.1038/natrevmats.2018.8
5. Gheysens, T., Beladjal, L., Gellynck, K., Van, N.E., Van, L.L. & Mertens, J. (2005). Egg sac structure of *Zygiella xnotata* (Arachnida. Araneidae). *J Arachnol,* 33,549–557.
6. Guarisco, H. (2001). Description of the egg sac of *Mimetus notius* (Araneae, Mimetidae) and a case of egg sac predation by *Phalacrotophora epeirae* (Diptera, Phoridae). *J. Arachnol*., 29, 267-269.
7. Huang, H.H., Ho, C.T., Lee, T.H., Lee, T.L., Liao, K.K. & Chen, F.L. (2004). Effect of

surface roughness of ground titanium on initial cell adhesion. *Biomol. Eng*., 21, 93-97.

8. Li, S.F.Y., Mcghie, A.J. & Tang, S.L. (1994). New internal structure of spider dragline silk melamine fiber reinforced EPDM rubber. *J. Mat. Sci*., 36, 2621-2632.

9. Rajeev, R.S., De, S.K. & Bhowmick, A.K. (2001). Atomic force microscopy studies of short melamine fiber reinforced EPDM rubber. *Jour. Mat. Sci*., 36, 2621-2632.

10. Trancik, J.E., Czernuszka, J.T., Bell, F.I. & Viney, C. (2006). Nanostructural features of a spider dragline silk as revealed by electron and x-ray diffraction studies. *Polymer*, 47, 5633-5642.

11. Zhao, A., Zhao, T., SiMa, Y., Zhang, Y., Nakagaki, K., Miao,Y., Shiomi, K., Kajiura, Z., Nagata, Y. & Nakagaki, M. (2005). Unique Molecular architecture of egg case silk protein in a spider, *Nephila clavata. J. Biochem.,* 138, 593-604.

12. Zinger, O., Anselme, K., Denzer, A., Habersetzer, P., Wieland, M., Jeanfils, J., Hardouin, P. & Landolt, D. (2004). Time dependent morphology and adhesion of osteoblastic cells on titanium model surfaces featuring scale resolved topography. *Biomaterials*, 25, 2695-2711