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Cylindrical Glands and Egg Sac Silk of Giant Wood Spider, *Nephila pilipes*

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Abstract: *Different types of silk glands are located inside the abdomen of spider. Three pairs of cylindrical glands are developed in female giant wood spider, Nephila pilipes. In the present study, attempt have been made to explain the anatomy and histology of cylindrical glands and details of its spinning field with the help of scanning electron micrograph (SEM). The microscale topology and surface nanostructure of egg sac silk threads was studied with advance techniques of atomic force microscopy (AFM).It is concluded that, in female spider, cylindrical glands and egg sac silk produced by them plays a very important role in successful perpetuation of species. Roughness analysis of egg sac silk suggested that this biomaterial has high toughness that may be suitable for dissipating high amounts of mechanical energy. This silk is having high and stable mechanical performance. Hence, successful large scale production of this beautiful and strong, naturally pink colored spider silk will definitely open a new gate in textile sector.*

Keywords: Nephila Pilipes, Cylindrical Glands, Egg Sac Silk, SEM, AFM.

I. Introduction

Different silk producing glands in spiders functions as small biofactories. In giant wood spider *Nephila pilipes*, two kinds of paired ampullate, three pairs of cylindrical, two pairs of aggregate, one pair of flagelliform, numerous pyriform and aciniform glands are found. All these glands are mainly tubular, end blindly and store specific types of silk in liquid form. Each gland leads to specific silk secreting outlet i.e. spinnerets which opens to outside in the form of tiny spigots.

Spider silks are formed from spidroins, a family of repetitive structural proteins [1]. Egg sac silk creates a protective layer against the trauma, as well as against predators and parasites [2], providing an approximate microclimate mediating against temperature and humidity fluctuations for embryonic development, hatching and subsequent molting and durable shelter for spiderlings [3]. The silk fibers of egg sac possess particular mechanical properties that allow the creation of a particular three dimensional structure. 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 [4]. Hence, the surface roughness of egg sac silk fiber was observed and studied.

With the progress and development of science and technology, people's demand for superior performance materials is getting higher and higher, which puts forward higher requirements for the research work on natural spider silk bionic materials and related science. A large number of superior properties of spider silk bionic materials will replace traditional materials [5].

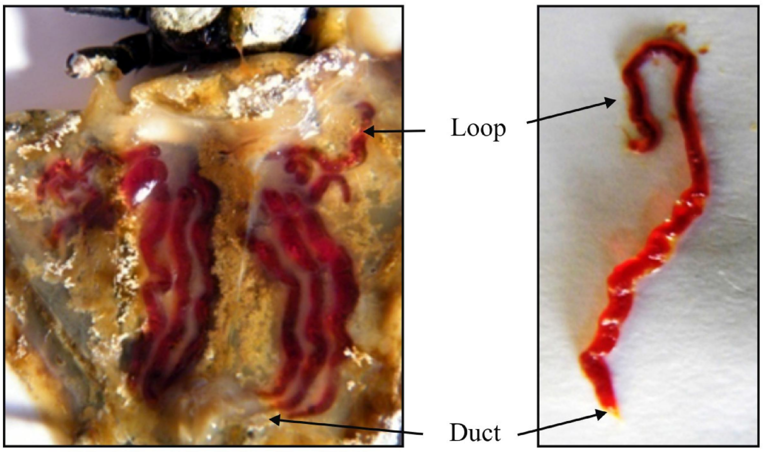
II. Materials and Methods

The adult females of *Nephila pilipes* spider was dissected under Magnus Steriozoom binacular Microscope with camera attachment. Photographs of cylindrical glands were taken with FUJIFILM FINEPIX 52000 HD 10.0 megapixel 15X wide digital camera. Glands were fixed in Bouins fluid (fixative), dehydrated through ethanol series and cleared in xylene. After cold and hot embedding, these glands were blocked in molten paraffin wax. These tissues were serially sectioned at 5 µm thickness using a rotary microtome. Tissues were stained with Hematoxylin and Eosin combinations and mounted in DPX for histological specifications. Spinneret of same spider was prepared for examination by SEM. Procedure was performed as described by Townley and Tillinghast [6] with a few exceptions. Spinneret was mounted on stub. Gold coating was done with Auto Fine Sputter Coater (Model No. JEDL JFC –1600) at current 20 mA and pressure 20 Pa. SEM photographs were taken on Digital Scanning Electron Microscope (Model No. JEOL JSM -6380A) at Visveswaraiya National Institute of Technology, Nagpur, India. The identification of spigots on spinnerets was done using rules given by Coddington [7]. 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.

III. Results and Discussion

Nephila pilipes possess highly elaborated arrangement of spinning glands. Three pairs of cylindrical glands are found along the ventrolateral sides of the body cavity and runs antero-posteriorly (Fig.1-a). The name cylindrical gland was employed because of cylindrical form of gland (Fig.1-b). The red coloured cylindrical glands are found only in adult female spider. Their size and secretory activity increases with the development of eggs in the ovaries [8]. The gland is uniform in diameter about 0.5-1mm. The anterior end bends in posterior direction to form a loop. Posteriorly, the gland continues into a duct that opens to exterior through a spigot with broad mouth. The gland appears red in colour due to red colour of the secretion. The morphology of gland is very much clear before the deposition of eggs by the female and diminishes in size after formation of egg sac. The pink colour of the silk used for egg sac confirms that the silk of these glands is employed for the construction of egg sac. The cylindrical glands do not refill, as the sole function of these glands is silk production for egg sac only.

(a)



(b)

(c)

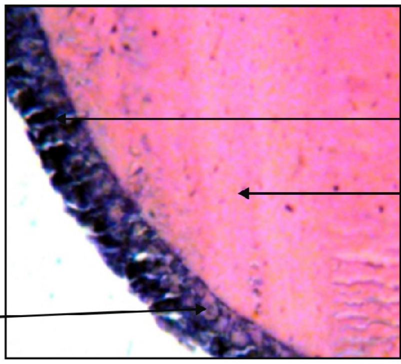


Figure 1: Morphology of cylindrical glands in *Nephila pilipes*

a. Three pairs of cylindrical glands in gravid female; b. Single cylindrical gland; c. T.S. of Cylindrical gland (H.E.X 400)

In transverse section (Fig.- 1 c), the gland appears to consists of a single layer of epithelial cells resting upon a thin basement membrane. The individual epithelial cell is columnar in shape. The nucleus is spherical and placed towards the base. The nucleoplasm appears granular. The cytoplasm around the nucleus is clear but that towards the lumen contains globules of secretion. Translational pauses have been detected in the secretory epithelium of cylindrical glands of *Nephila clavipes* [9]. In addition to the secretory globules, these epithelial cells contain brownish granules which are more concentrated near the base of the cell. The distal end of the epithelial cell is more conspicuous and a thin basement membrane lines the lumen. In addition to the epithelial cells, there are interstitial cells. These cells rest upon the basement membrane and have varying sizes. The nucleus of the interstitial cell strikingly differs from that of the epithelial cell in being more or less oval in shape. The cytoplasm does not take any stain and shows few vacuoles. The gland is externally covered by a thin but quite distinct membrane of connective tissue. The lumen of the gland is continued into the lumen of duct. The luminal contents stains pink with eosin.

Silk glands of *Nephila pilipes* open on three pairs of spinnerets. The terminal part of each spinneret is provided with spigots. The spigots of different glands are placed on different spinnerets have functionally reasonable ''upstream-downstream'' explanations. The cylindrical gland spigots are downstream with respect to other spigots [10].

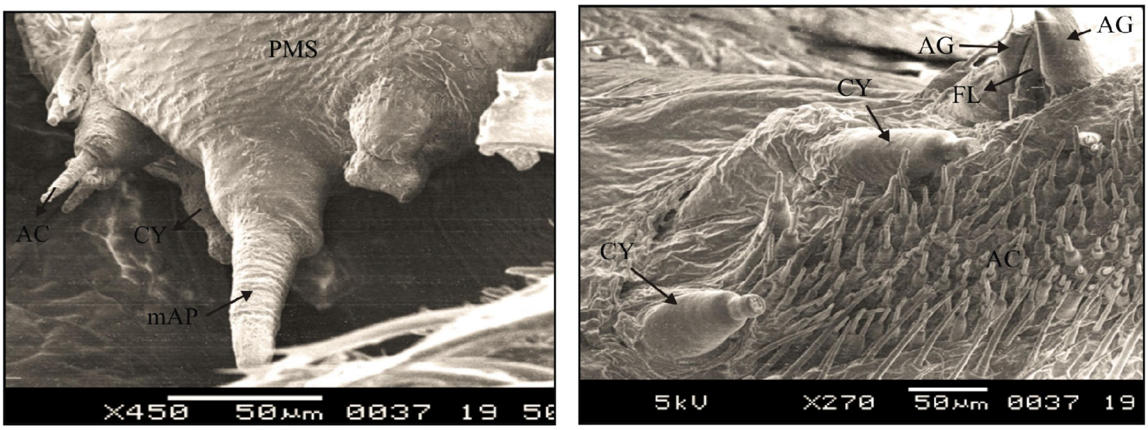


Figure 2: Spigots of cylindrical glands (CY) in *Nephila pilipes*

a. One spigot on PMS b. two spigots on PLS

One cylindrical gland opens on the posterior median spinneret (PMS) through single cylindrical gland spigot, CY (Fig.-2 a). The mesal basal margin of posterior lateral spinneret (PLS) shows two cylindrical gland spigots (Fig.-2 b). The spigots of the fastening silk like egg sac silk need to be downstream of the spigots that produce the silk lines which are being fastened.

The topography of egg sac silk was observed by using Atomic force microscope (AFM). The topography is not uniform. Patches of this silk thread showed alternate smoother and rougher surface. Such patches on egg sac silk threads were also observed under light microscope**.** For roughness analysis, three values 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. Hence, the measurements were performed using two different scan windows for egg sac silk (Fig.-3, Table -1). The white portion from the figure is the projection from base matrix [11]. 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 [12]. The peculiar topographical structure of spider silk may be due to presence of secondary structural elements like β-sheets, β-turns, α-helices etc [13]. These well organized features gives typical strength to spider silk. Gellynck *et al.* [14] gave simple treatment to makes this strong and flexible protein fiber ready to be used in many biomedical applications.

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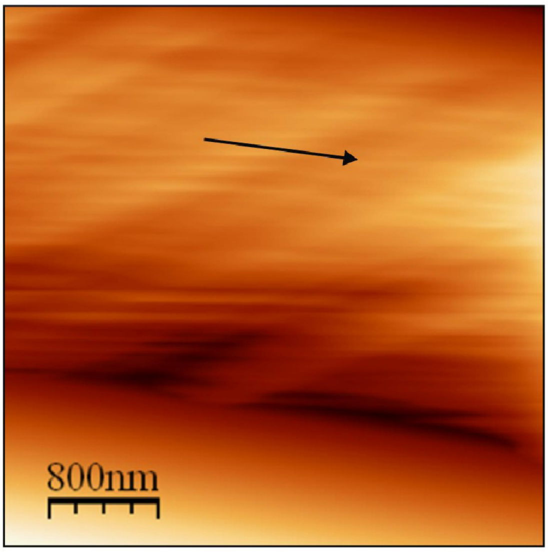
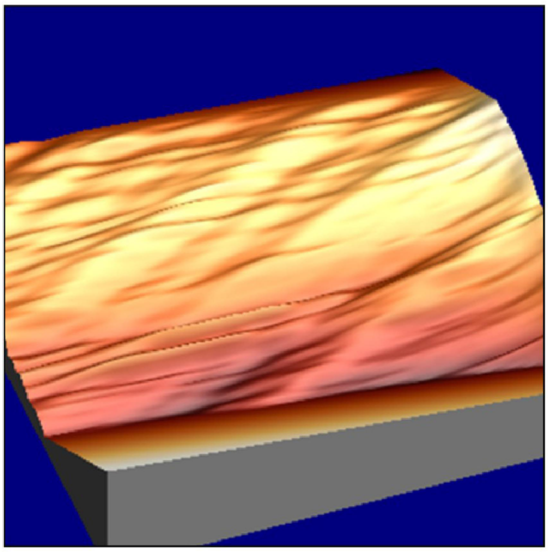
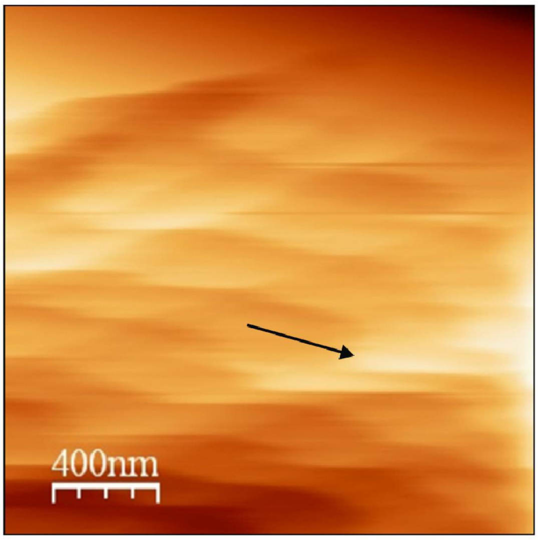
Table 1: Surface roughness, RMS, Ra and H for egg sac silk of *Nephila pilipes*

Scan Size	R.M.S.(nm)	Ra(nm)	H(nm)
4 x 4 μm^2	323.72	717.02	1770.79
2 x 2 μm^2	254.87	797.38	555.43



a


b



c

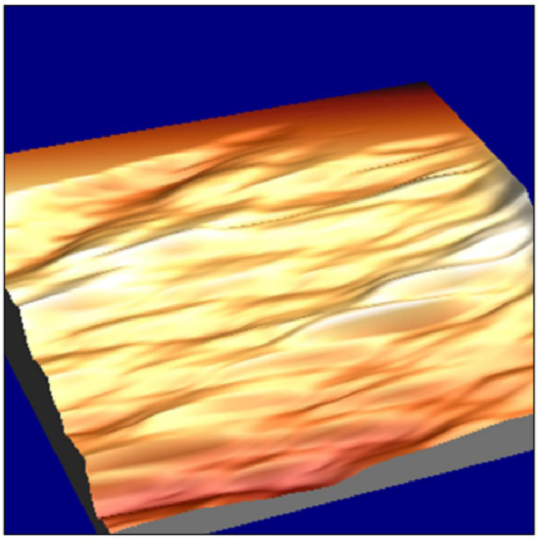

d

Figure 3: AFM images showing topography 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

IV. Conclusion

Cylindrical glands and egg sac silk produced by female spider in their natural habitat plays a very important role in successful perpetuation of species. Roughness analysis of egg sac silk shows that this silk has high toughness and displayed strong mechanical properties. Hence, successful large scale production of this beautiful and strong, naturally pink colored spider silk will definitely lead to development of novel biofibers, which can open a new gate in textile sector.

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