EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 2 (February-2015) pp. 20-27

www.ijcrar.com

Ultra structural study of *Mytilus viridis* byssus through scanning electron microscopy

M.B.Patel and K.A.Pithawala*

Department of Biology, Gujarat Arts and Science College, Ellis bridge, Ahmedabad-380006, Gujarat, India

Corresponding author

KEYWORDS

Mytilus viridis, scanning electron microscope, SEM, byssus

A B S T R A C T

Both coated and uncoated byssi when observed under SEM at various magnifications revealed unique structures (corrugated contours, broken and peeled surfaces). The byssus differed in the surface topography along its entire length. Bends and breaks were seen distinctly differing in their structures. The old and new byssi differed in their texture. The portion lying inside the gland appeared like stacks of plates. It had a regular symmetry from which the individual byssus emerged. The peculiar observation was that some threads were thinner, finer and had no cylindrical structure; instead they showed a unique structure consisting of stacks of cups one over another - an appearance similar to serrate antennae of insects or strobilus. The detailed SEM studies could through some light on the intermediate stages during the development of byssus. Internal exposed structure showed honeycomb appearance. This particular structure can be accounted for extraordinary strength and adhesive property of the fiber.

Introduction

Mussels are distributed in marine and estuarine regions of India, copiously on the coasts of Maharastra, Goa, Karnataka, Kerala and Tamilnadu (Trivedi *et al.*, 1986). Green mussels [*Mytilus (Perna) viridis*] have been recorded from the shores of Dwarka, Mocha and Okha along the Gujarat coast (Tiwari *et al.*, 2000). The genus is characterized by the presence of single median foot which houses a byssus gland and a byssus groove. The former secretes a unique protein called ‘byssus’ protein that is known to adhere in aqueous medium and provides a firm anchorage to the animal and prevents its dislodgement under various stresses and strong tidal waves or water currents (Field, 1922).

Byssus is known since time immemorial. People of Egypt and oriental regions used to weave cloth out of byssus and since it had fine structure it became the most costly fabric in the ancient world. It has been found in the tombs of Egyptian Pharaohs, and is often mentioned in the Bible, where it is said to be obligatory for the carpets of the Holy of Holies and for the "Ephod", the vestment of the high priests (Shamireaders, 2005).

The biological origin of this glue and the ability to stick to nearly all surfaces invite applications such as the development of surgical adhesives (White, 1937). The fact that the adhesive can stick opportunistically to any hard surface even teflon coated surfaces makes this an excellent research material (Ruppert *et al.*, 2004 and Bairati and Zuccarello, 1974).

This paper describes a detailed examination of byssus threads (old as well as newly formed) to understand their external contours which can be utilized in biotechnological applications. Also a study on the internal structure of thread has been carried out in order to throw some light on its adhesive properties, strength and developmental stages of threads.

Materials and Methods

Both mature and immature byssus threads were collected from the animals maintained in laboratory condition. They were washed first with tap water several times and then washed thrice with distilled water. The threads were then dried completely and used for SEM studies. Uncoated as well as gold coated threads were used. Environment Scanning Electron Microscope (ESEM) studies of mature and immature byssus threads were done without coating at SICART (Sophisticated Instrumentation Center for Applied Research and Testing), Anand, India). At the same time byssi were gold coated by sputter coater (Fision SC 7610 Quorum Tech. UK.) at the facilitation center of IPR (Institute of Plasma Research), Gandhi Nagar, India. The sections of these gold coated byssi were taken for scanning electron microscope (LEO S-440i, Cambridge UK) study.

For SEM studies, the following three regions of the mature and immature byssus threads were selected (i) Plaque (tip) (ii) the thread i.e. middle region and (ii) the root attached to the gland.

Results and Discussion

The plaque (Fig. 1) had a distinct structure whose surface was smooth as compared to the thread (Fig. 2). Not much difference was observed between mature and immature byssi plaques but the thread and root of the byssi had large differences. The mature threads had rough surfaces with many contours (Fig. 3) and the root of the mature threads was curved (Fig. 4) rough in texture and easily differentiated from immature byssus.

The main aim behind studying the structure of threads at SEM level was to find out whether the threads could be utilized in biotechnological applications such as immobilization of enzymes etc.

When a bunch of byssi was observed, the portion which was inside the gland looked like stacks of plates and had a regular symmetry from which the individual byssus emerged (Fig. 5a–c). This structure is distinguishing characteristic of the species. The regions of bending (Fig. 6) and twists (Fig. 7) give an idea of internal fibrous nature of the byssus. Breaks (Fig. 8) show that a thread, though being flexible in nature of movement, is still rigid enough with tensile strength.

One interesting observation (Fig. 9a) was that some threads, which were thinner and finer, had no cylindrical structures. Instead, they showed a unique structure consisting of stacks of cups one over the other, an appearance, very much looking like the serrate antennae of insects or like strobilus

(Fig. 9b). Certain threads, bit thicker than the former (Fig. 10) had similar arrangements but the cups were placed in such a way that they merged into one another leaving scales at rims of the cups (Fig. 11). Such a structure can be explained as an intermediate stage in the development of byssus. This however needs further experimentation for confirmation.

In one of the SEM studies (Fig. 12), byssus thread preparation was such that its surface skin was peeled off and internal structure was exposed. To the best of our knowledge, these features have not been reported so far. Here, the internal anatomy showed honeycomb like structures but facets of the cell were not hexagonal. Instead they were more rounded looking like a cluster of swift's nest, sometimes coral or sucker like at higher magnifications (Fig. 13 and 14). This particular resin structure can be accounted for extraordinary strength of the fiber plus its adhesive property.

Bairati and Zuccarello (1974) have carried out detailed investigation with scanning electron microscope (SEM) of surface morphology of the byssus of *Mytilus galloprovincialis.* Further they also studied internal structure by microdissection with the freezing microtome. They reported that the stem could be regarded as an organ that undergoes extensive age-related structural changes, chiefly consisting of variations in the number and arrangement of its threads. The stem and the thread differ considerably in structure, as expected from their different mechanical function. The stem consists of an outer layer of laminar "cuffs" wrapped around a cylindrical "core". The threads are made up of different portions, i.e., (1) a rigid portion (cuff), by which they are attached to the stem; (2) a straight, elastic proximal portion where a corrugated sheath envelops a central rectilinear core; (3) a rigid, cylindrical distal portion; (4) an adhesive plate which anchors to the substratum (Fig. 15).

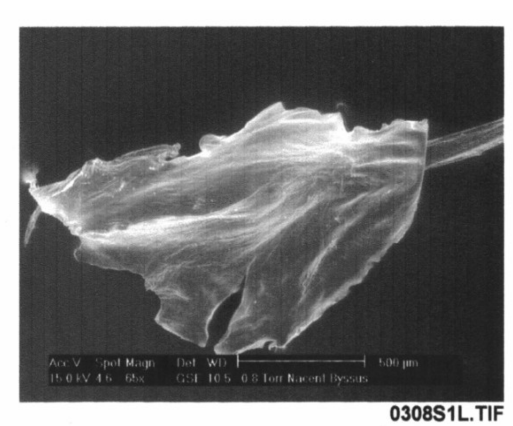

Fig.1 SEM of Plaque of byssus of *M. viridis* showing smooth surface contours (64X)

Fig.2 SEM of byssus thread stem showing rough contours (2500X)

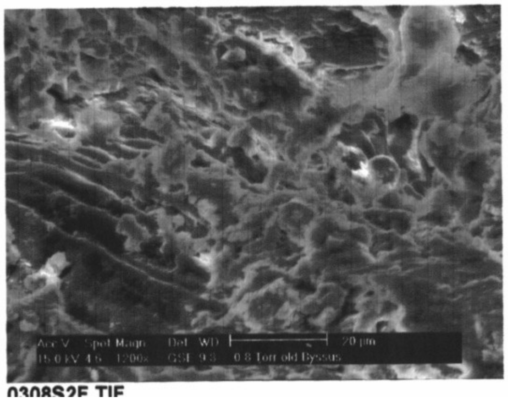

Fig.3 Surface of mature byssus thread showing many contours (1200X)

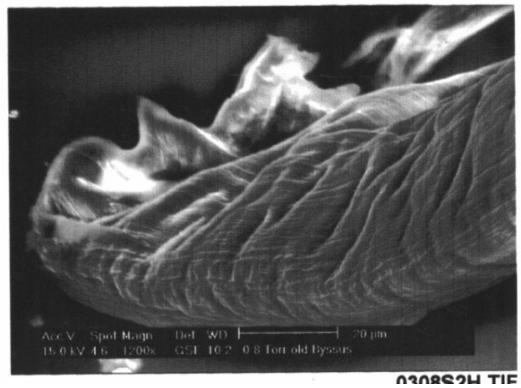

Fig.4 Curved end of mature byssus thread as seen under SEM (1200X)

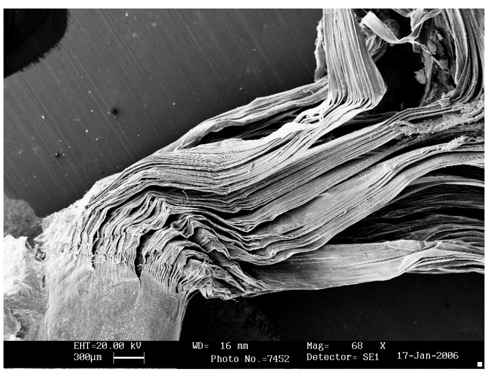

Fig.5a Cuff of byssus showing plaque like structure (64X)

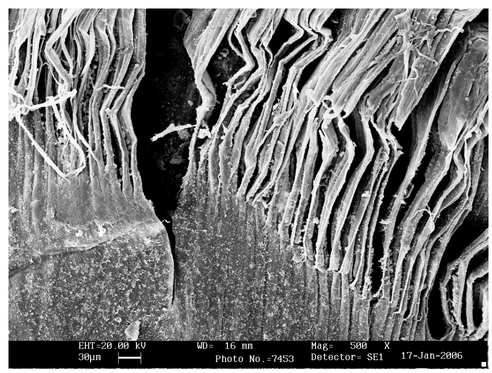

Fig.5b Cuff of byssus showing plaque like structure (500X)

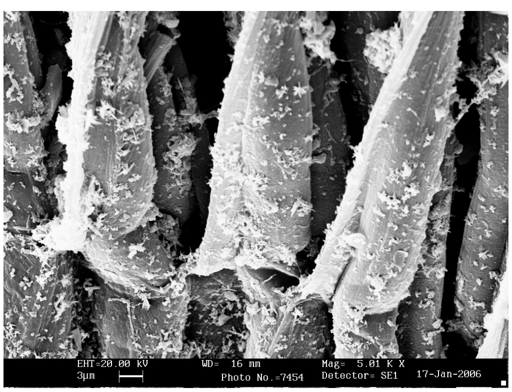

Fig.5c Plates of the cuff of byssus highly magnified under SEM (5010X)

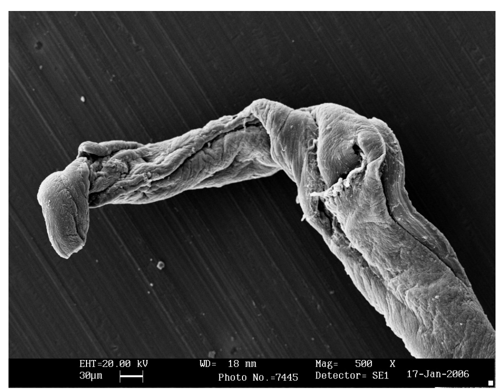

Fig.6 SEM of byssus threads showing regions of bending (500X)

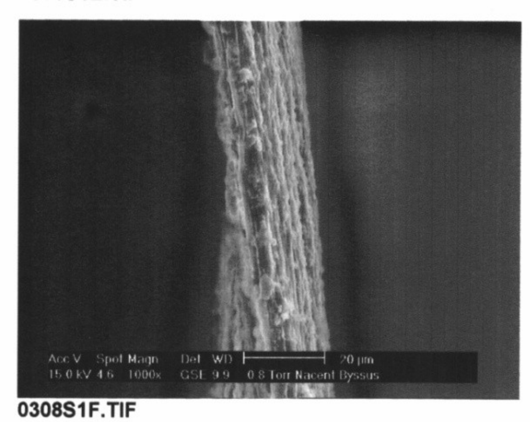

Fig.7 SEM of byssus thread showing regions where there is a twist (1000X)

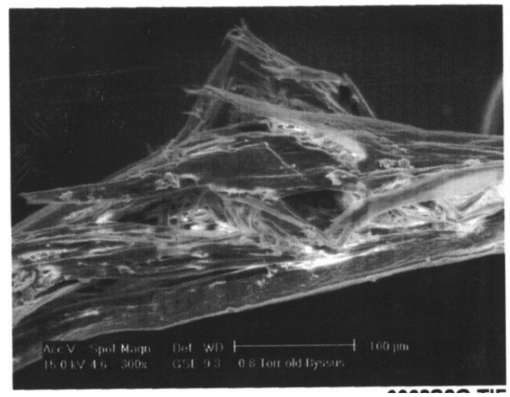

Fig.8 SEM of byssus thread showing broken region (300X)

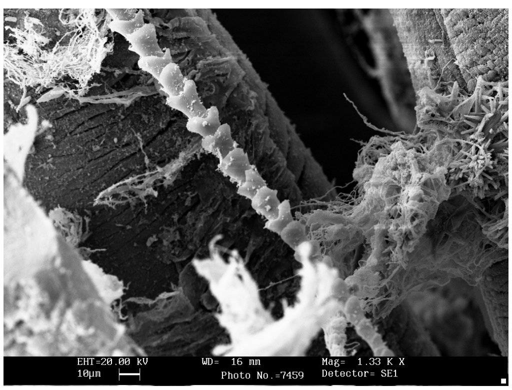

Fig.9a SEM of byssus threads showing one nascent byssus appearing like the serrate antennae of insects (1330X)

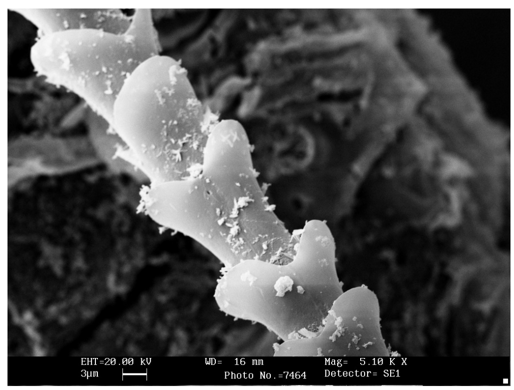

Fig.9b SEM of byssus threads showing one nascent byssus appearing like the serrate antennae of insects (5100X)

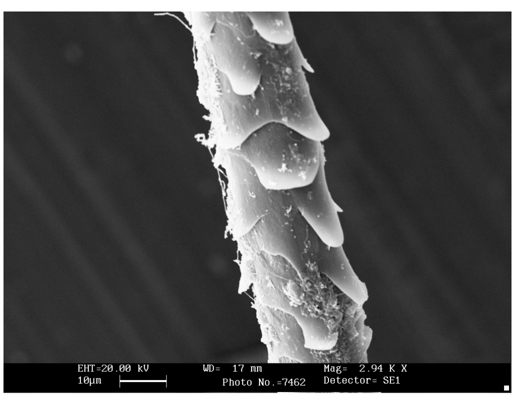

Fig.10 SEM of fine byssus threads showing scales merging into the main stem (2940X)

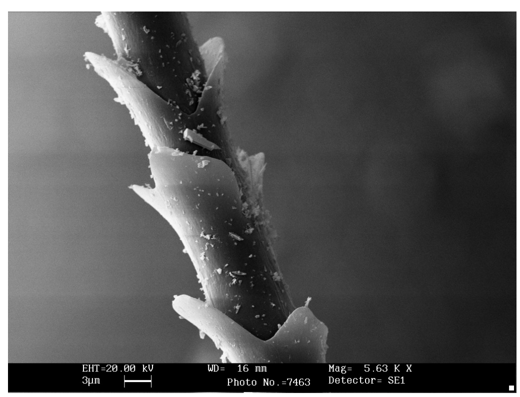

Fig.11 SEM of fine byssus threads showing scales beginning to merge into the main stem (5630X)

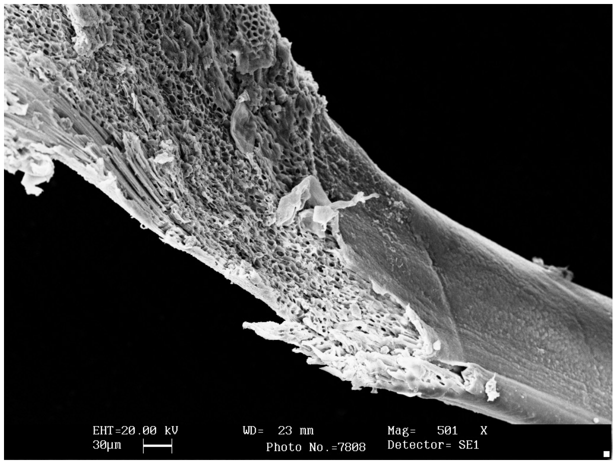

Fig.12 SEM of peeled byssus thread showing its internal honeycomb like structure (500X)

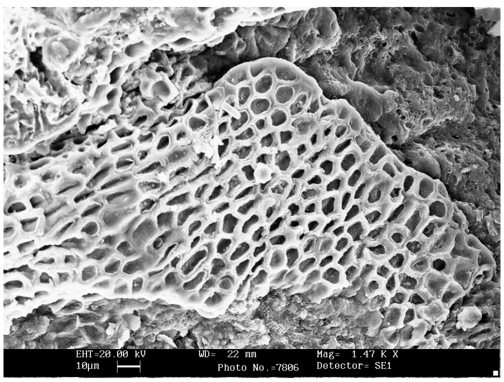

Fig.13 SEM of peeled byssus thread showing its internal coral like structure (1470X)

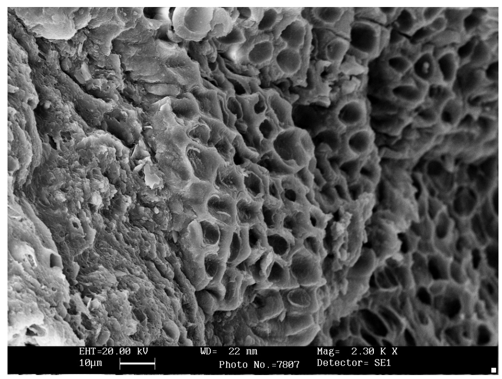

Fig.14 SEM of peeled byssus thread showing its internal swift-nest like structure (2300X)

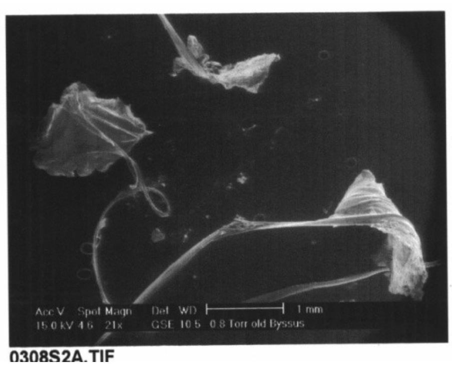

Fig.15 SEM of byssi showing different regions of plaque, stem and root (21X)

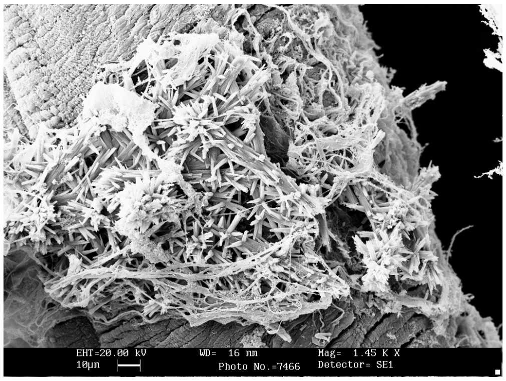

Fig.16 SEM of byssus material secreted out of the gland appearing like crystals and fibres (1450X)

Fig.17 SEM of byssus material secreted out of the gland appearing like crystals and fibres (5010X)

Fig.18 SEM of byssus material secreted out of the gland and has aligned in a fan like fashion (11040X)

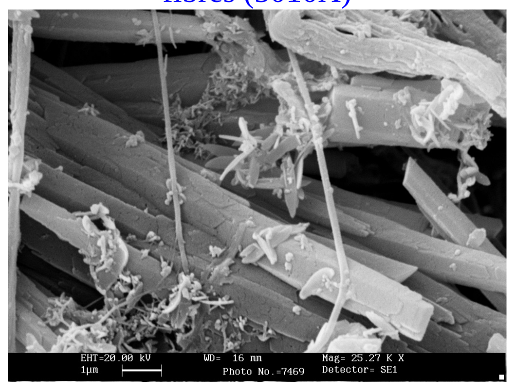

Fig.19 SEM of byssus material secreted out of the gland and has aligned in a linear fashion one after another in stacks (25270X)

In another SEM study of byssus threads, a view of byssus material that was secreted outside the gland was captured (Fig. 16). Here, the material appeared to have two types of structures. One was regularly arranged array of crystals of various sizes but similar shapes (Fig. 17); while another structure showed few orthogonally placed fibers to the crystals.

The same material at nearby location when magnified showed that the crystals had aligned one after another and were in stacks. The points of ligatures were clearly seen. Further, these stacks were wound around by fibers (Fig. 19).

The present study is an attempt to understand the mechanism of byssus thread formation. It is evident that the byssus structure, external as well internal is not uniform along the entire length from plaque to the root because byssus thread is secreted by various glands situated in and along the byssus groove of the foot and the length of the extended foot during its secretion determines the length of the byssus thread.

Acknowledgement

The authors are grateful to U.G.C. New Delhi, for financial support.

References

Bairati, A., Zuccarello, L.V. 1974. The ultrastructure of the byssal apparatus of *Mytilus galloprovincialis*: II. Observations by microdissection and scanning electron microscopy. *Mar. Biol.*, 28: 145–159.

Field, I.A. 1922. Biology and economic value of the sea mussel, *Mytilus edulis*. *Bull. Bur. Fish.*, 38: 127–259.

Ruppert, E.E., Fox, R.S., Barnes, R.B. 2004. A functional evolutionary approach. In: Brooks, C.T., Belmont, C.A. (Eds). Invertebrate zoology. Brooks Cole Thomson, Belmont, CA. 963 Pp.

Shamireaders, January 6, 2005. The true face of Jesus Paul Badde, 29 September 2004 (Feast of the Archangels Michael, Gabriel & Raphael) Courtesy Israel Shamir.

Tiwari, A., Joshi, H.V., Raghunathan, C., Shrankumar, V.G., Koliwar, O.S. 2000. New record of *M. viridis* (Linn), Its density, growth and accumulation of heavy metals on the Saurastra coast, Arabian sea. *Curr. Sci.*, 78(1): 97–101.

Trivedi, C.R., Bhaskaran, M., Wadher, D.J., Khhayya, F.D. 1986. Successful long distance road transport of green mussel from Calicut to Port Okha. Indian Counc. Agricult. *Res. Mar. Fish. Inf. Serv. Tech. Exten. Ser.*, 24, No. 70.

White, K.M. 1937. *Mytilus*. Liverpool Marine Biol. Committee. 311-17, 1-30.