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Ultrastructural Studies on Byssus Gland Cells of *Mytilus Viridis* through Transmission Electron Microscopy

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Abstract— TEM of byssus gland cells reveal unique vesicles at various stages of development and coalescence. The cell shows a distinct nucleus as well as other cell organelles viz. mitochondria and endoplasmic reticulum. Golgi body could be seen, but what was more interesting was that there are numerous secretory granules which when seen under higher magnification show fibrous structures. Moreover those vesicles that burst were surrounded by fibrous material; this can be accounted to be protein of byssus. Rough ER was not seen.
Keywords— Mytilus, viridis, Perna, byssus, Transmission Electron Microscopy.

I. INTRODUCTION

Mytilus or the sea mussel is a marine animal of cosmopolitan distribution belonging to class pelecypoda of phylum mollusca [1]. It is sedentary animal lying between low tide marks and filter feeding on diatoms, protozoa, bacteria and organic detritus. The family mytilidae to which the genus *Mytilus* (Linn. 1758) belongs is believed to have its origin as far back as Devonian era some 400 million years ago [2]. The animal has many species and is having world wide distribution. In India *Mytilus viridis* (Linn), also called *Perna viridis* (green mussel) is found to be distributed along the southern coasts [3]. It supports the substantial fishery of some consequence along the coast of Karnataka and Kerala. Though green mussels are widely distributed in marine and estuarine regions of India, they are fairly abundant on the coast of Maharashtra, Goa, Karnataka, Kerala and Tamilnadu [4]. However; green mussel has also been recorded from the shores of Dwarka, Mocha and Okha along Gujarat coast [5]. The genus is characterized by the presence of single median foot which houses a byssus gland and a byssus groove, the former is known to elaborate a unique protein called byssus protein 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 [6]. The biological origin of this glue and the ability to stick to nearly all surfaces invite applications such as the development of surgical adhesives [7].The fact that the adhesive can stick opportunistically to any hard surface even Teflon coated surfaces make this an excellent research material [8-10]. Scientists are dissecting natural adhesives into their tiniest components, while searching for the underlying rules that will make sense of the data being uncovered in their laboratory. According to some workers foot consists of some six glands [11], all involved in the production of byssus and thus it is clear that byssus is a complex structure and different in nature of composition as well as function and can be differentiated into three regions viz. an adhesive plaque, a middle thread and a proximal root all continuous with each other without any line of demarcation [12]. One of the great mysteries driving scientists involves the cross-linking that occurs within the adhesive, specifically the way this protein folds in upon itself. Current chemical understanding dictates that protein folding occurs in the middle of a molecule, which would draw the material together, minimizing its interface with the surface and, consequently, reducing adherence. But this natural superglue does the opposite. It spreads out, maximizing surface-to-volume ratio. This is queer behavior and not shown by other proteins. The explanation will depend on coming up with new rules to explain the behavior of this protein. . The researches that are being conducted will eventually produce effective models to explain the actions of these macromolecules. Once this is explored it will allow people to make effective adhesive molecules, as well as the enzymes needed to synthesize them. Producing these natural superglues in bulk might be a great help to the medical device industry [13]. Researchers in this field might learn enough from the natural processes to build a "peptide maker" that could produce quantities of these glues on demand. Among the leading candidates is yeast and virally infected insect cell lines. The infected insect cells attract scientists the most because they have many of the enzymes that appear to be needed to do the processing that gives the adhesive proteins their requisite "stickiness" [14]. Exactly when such molecular production lines will be available, however, are difficult to predict. Scientists believe science may be able to imitate the adhesion of marine invertebrates within coming years. The proteins and partial strategies that come from

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these can frequently have very useful spin-offs that can be applied without knowing the whole answer. In this paper studies on the TEM of the gland cells is done to get more insight into the mechanism of byssus production by knowing the secretion of byssus precursor at the cellular and subcellular level. In addition, an idea about the detailed structure of the thread can be had for its further implications and usages.

II. MATERIALS AND METHOD

A. Preparation Of Material For TEM

Foot was excised from mussel and fixed in cold 5% glutaraldehyde in 0.01 M phosphate buffer for one hour. After washing in buffer, the tissue was post-fixed in 1% osmium tetroxide for an hour, dehydrated in a graded ethanol series and infiltrated as mentioned by Bairati and Zuccarello (1974) [15] . This was then embedded in Spurr's medium. Thin sections were stained with uranyl acetate followed by lead citrate. The sections were examined with a Zeiss (Germany) Transmission Electron Microscope.

III. RESULTS AND DISCUSSION

The TEM of the gland was done to study the secretory product at the sub cellular level. The cells and a distinct nucleus as well as other cell organelles like mitochondria and endoplasmic reticulum, Golgi body could be seen but more interesting was that there are numerous secretory granules (Plate-1). When these secretory granules were seen under higher magnification (Plate-2), they showed fibrous structures. Moreover those that burst were surrounded by fibrous material. This can be accounted to be protein of byssus (Plate-3 to 6). Also vesicles at various stages of development, coalescence, empty and partially filled conditions throw some light on the mechanism of secretion of byssus material. Vesicles at the stages of formation appear completely dark and are of various sizes (Plate-7 and 8). Also vesicles which have poured their secretions appear empty (Plate-9). These empty vesicles come very close to each other and appear to be coalescing (Plate-10). Some of the vesicles appear to be half filled with secretion as they have poured out some material (Plate-11). The fibrous materials poured out appear in two forms one as stacks of straight fibers (Plate-12 and 13) and another as bundles of fibers with fringed margins (Plate-14). One peculiar examination is that those vesicles that pour out their secretions become irregular in their outer margins and are mainly located near the nucleus (Plate-15). The fibrous material (appearing as crystals) in some of the cells appear to align themselves in a particular fan like fashion (Plate-16). Such a growth of crystal of fibrous material is also seen during tissue culture studies [16]. This phenomenon has also been reported by the author on SEM studies of the byssus [17].

An understanding of this transformation might come from transmission electron micrographs being taken by Thomas Bonner, PhD, professor of biology at SUNY Brockport [18]. Bonner's micrographs show the adhesive as granules of glue resting beside granules of the enzyme, a type of catechol oxidase. Evidence suggests that these granules open up and merge. An understanding of how this process occurs may come from a detailed analysis of the extraordinarily complex structure of these granules, as well as from studies of material nature of byssus.

The Aluminium-formaldehyde (ALFA) histofluorescence method carried out by other scientists [19] revealed an extensive plexus of brilliant greenish monoaminergic elements in the glandular zones of the *Mytilus* foot, while only scanty nerve fibers were acetyl cholinesterase-positive. By electron microscopy, bundles of nerve fibers can be seen in close connection with the intrinsic musculature located in the connective septa among the glands, and near the cell bodies and necks of all the byssus glands. The nerve fibers show varicosities containing three types of vesicles: small clear (50–60 nm), small granular (80–90 nm), and large granular (160–200 nm). The regions of close apposition between nerve terminals and muscle or gland cells generally do not show typical pre- or postsynaptic specializations. Along the pedal groove, mainly in the proximal two third of the foot, peripheral bipolar neurons can be detected, both by fluorescence and electron microscopy. In our studies, these features were not observed. Electron micrograph allows us to see the way the byssus is organized at a high level of magnification, and then we get to look at the interface between the byssus and the substrate to understand the roles they play. The byssus is outside the living cells that compose the animal's foot, and is the mussel's equivalent of fingernails. Both the byssus and human fingernails are composed of protein. But unlike fingernails, the byssus forms tethers, called byssus threads. The adhesive connects the byssus to the underwater surface, and the byssus tethers the mussel to the surface. Studies at SUNY Brockport indicate that after the granules become part of the byssus and participate in the attachment process—the actual gluing—they are transformed into a structure composed of very densely packed filaments [20]. Still evolving is an understanding of the role played by the biofilm that coats the surface being attached. This slime is made of living organisms, primarily bacteria, some algae, and perhaps fungi, along with decomposing organic material. There is preliminary

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evidence that the byssus is initially released in a semi-liquid or gel state, which then flows into the interstices of the biofilm and solidifies, trapping parts of the biofilm in the byssus itself, the reason one thinks this is that one finds a fair number of bacteria trapped in the bottom of the byssus where it interacts with the surface. Marine animals such as the New England blue mussel also secrete a byssus and threads, but in these animals the threads are elastic and serve to absorb the force of the water as it rushes past. These threads elongate and then recoil to their original position when the force is removed. This natural "shock absorber" enhances the animals' ability to adhere to the surface and may provide a model for developers of medical adhesives [13].

It is observed through TEM studies that some of the byssus material within the cell has a crystal like structure. Therefore, in the case of *Mytilus viridis* the threads are not stretchable. They serve the purpose of permanent anchorage. Byssus gland cells show numerous vesicles (electron opaque) at various stages of development and coalescence. These vesicles when observed under higher magnification show presence of fibrous material contained in them. Vesicles that have emptied their secretion appear irregular in outline and lie near the nucleus. The fibrous material secreted out of the vesicles appear in two forms as stacks of straight fibers and as bundle of fibers with fringed margins. Some fibers are seen to align in a fan like fashion. And appear crystal like in structure. Byssus threads are not stretchable.

IV. ACKNOWLEDGEMENT

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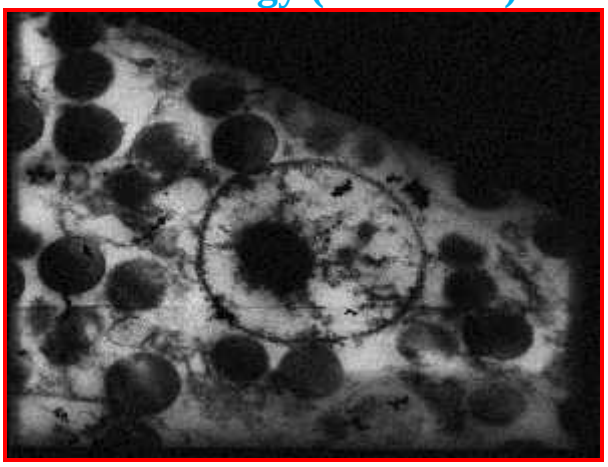


Plate-1 -TEM of byssus gland cell showing nucleus and numerous secretory vesicles. (7000X)

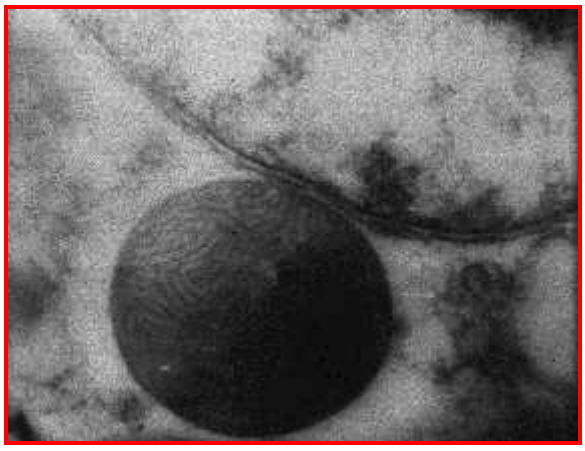


Plate-2 -TEM of byssus gland cell-vesicle enlarged showing fibrous content. (15000X)

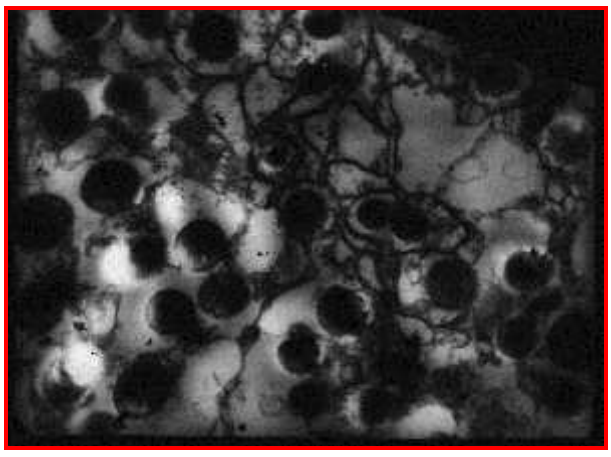


Plate-3-TEM of byssus gland cell showing vesicles with secreted fibrous products. (7000X)

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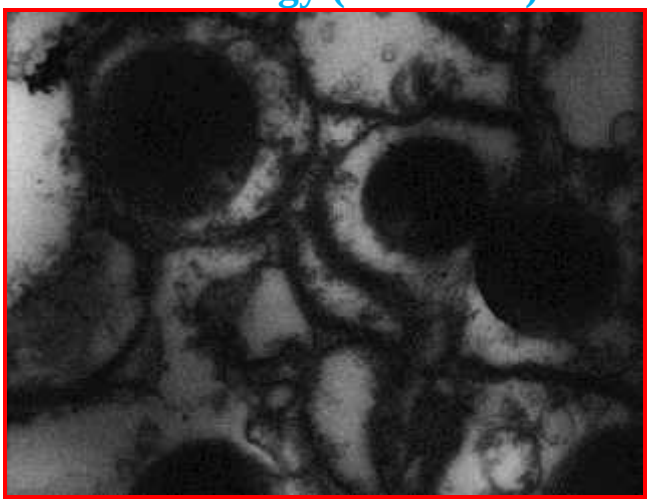


Plate-4-TEM of byssus gland cell showing vesicles with secreted fibrous products magnified. (15000X)

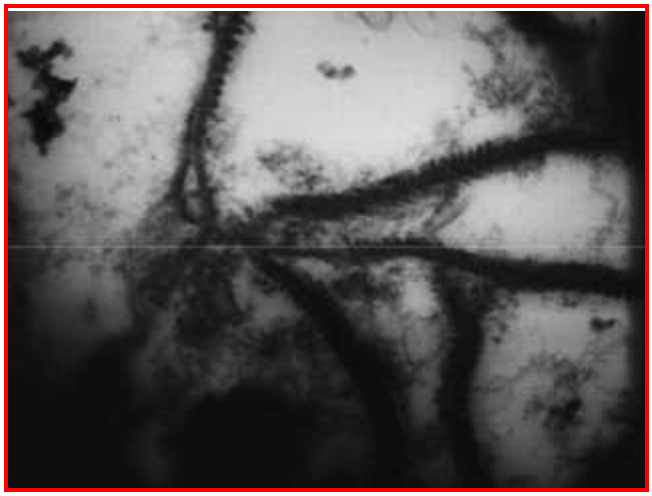


Plate-5 -TEM of the cell showing the secretory fibrous material highly magnified. (28000X)

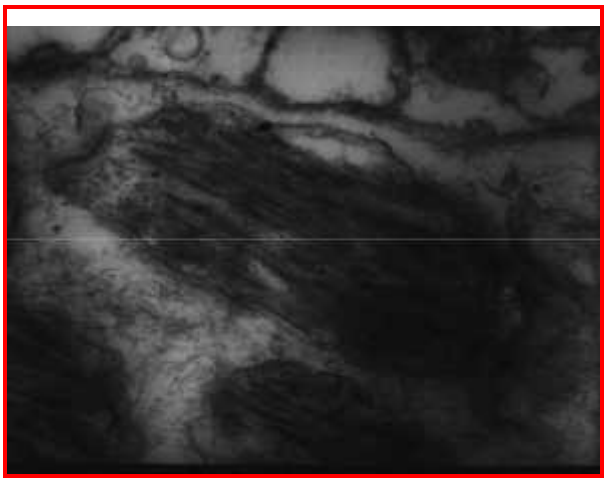


Plate-6 -TEM of fibrous material of the vesicle magnified. (28000X)

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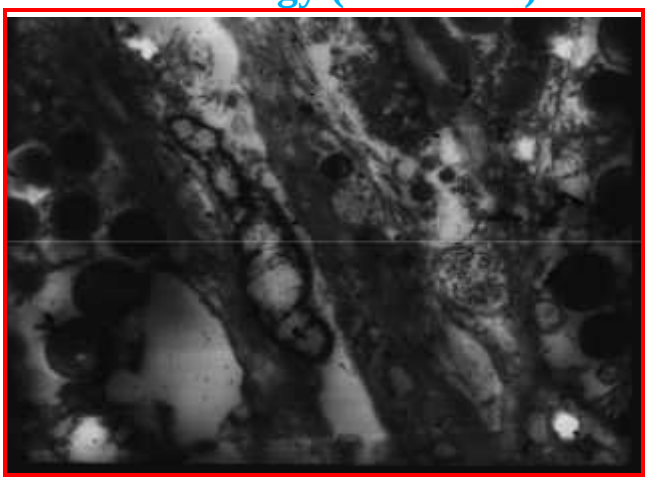


Plate-7 -TEM of vesicles at various stages of development. (7000X)

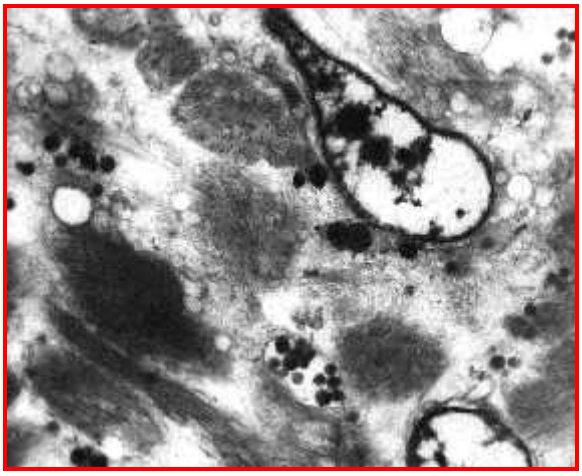


Plate-8 -TEM of secretory materials and vesicles at the stages of development. (11000X)

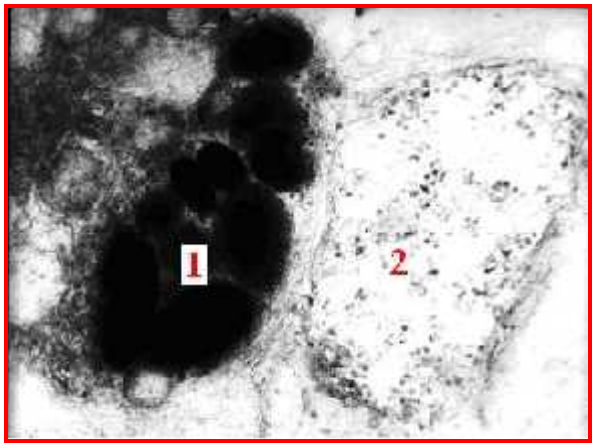


Plate-9 -TEM of coalesced vesicles, 1- filled with secretory material, 2- emptied. (7000X)

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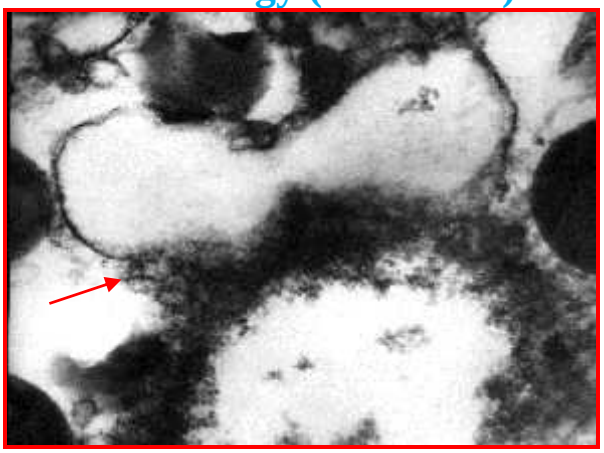


Plate-10- TEM showing empty vesicles that show coalescence. (11000X)

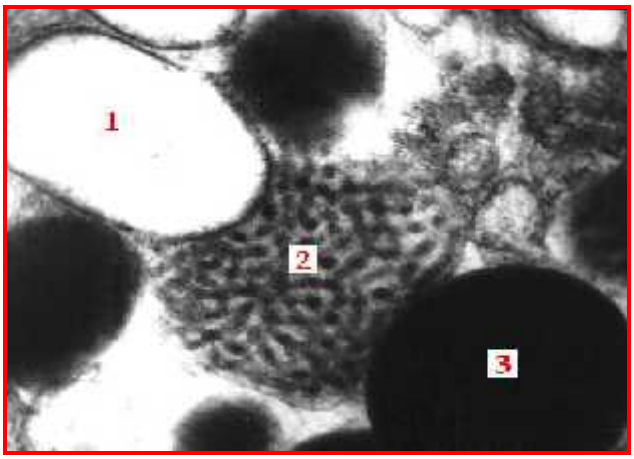


Plate-11- TEM of vesicle at intermediate stage of secretion, 1- empty, 2- half filled and 3- completely filled. (18000X)

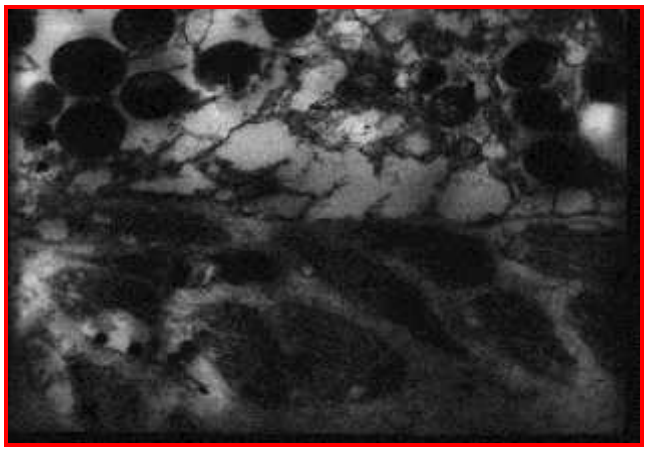


Plate-12 -TEM of vesicles at various stages of secretion of fibrous material. (7000X)

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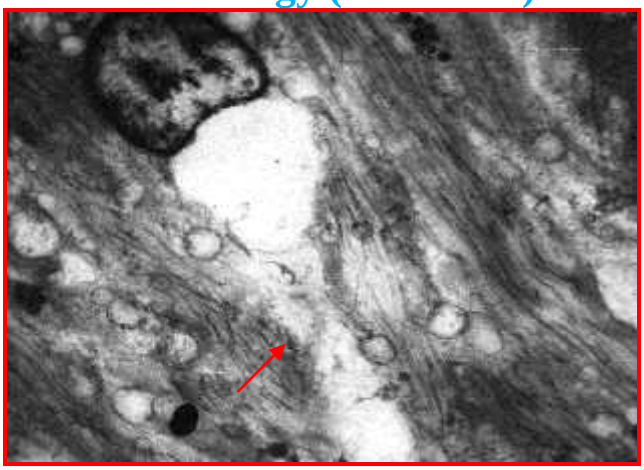


Plate-13 -TEM of secretory fibrous material aligning them as stacks. (20000X)

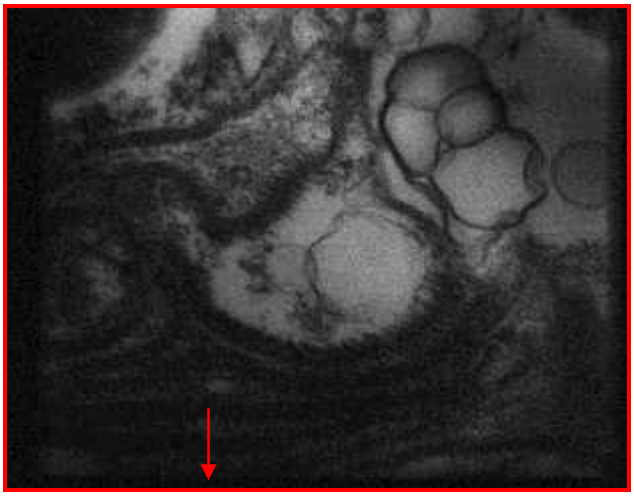


Plate-14 -TEM of vesicles that have been emptied after pouring off their secretory material the fibrous material appears as bundles of fibers with fringed margins. (15000X)

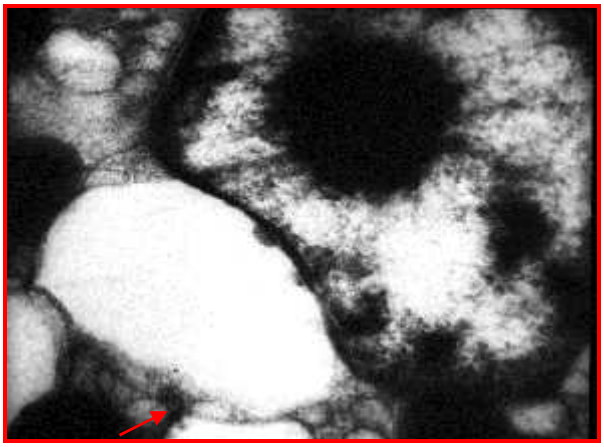


Plate-15 -TEM of emptied vesicle seen near the nucleus whose membrane has been pushed inside. (11000X)

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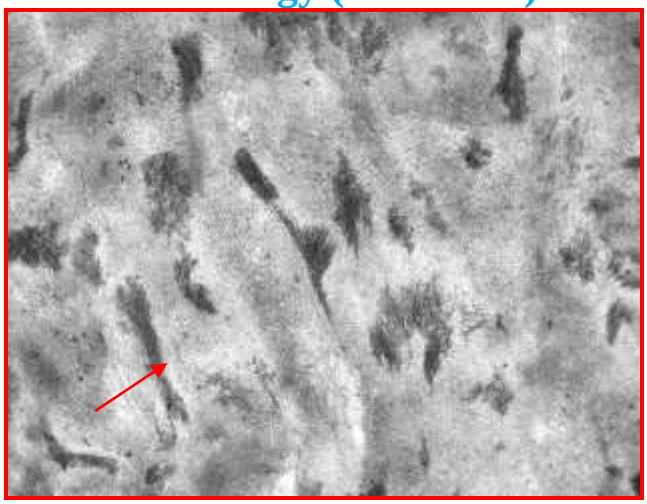
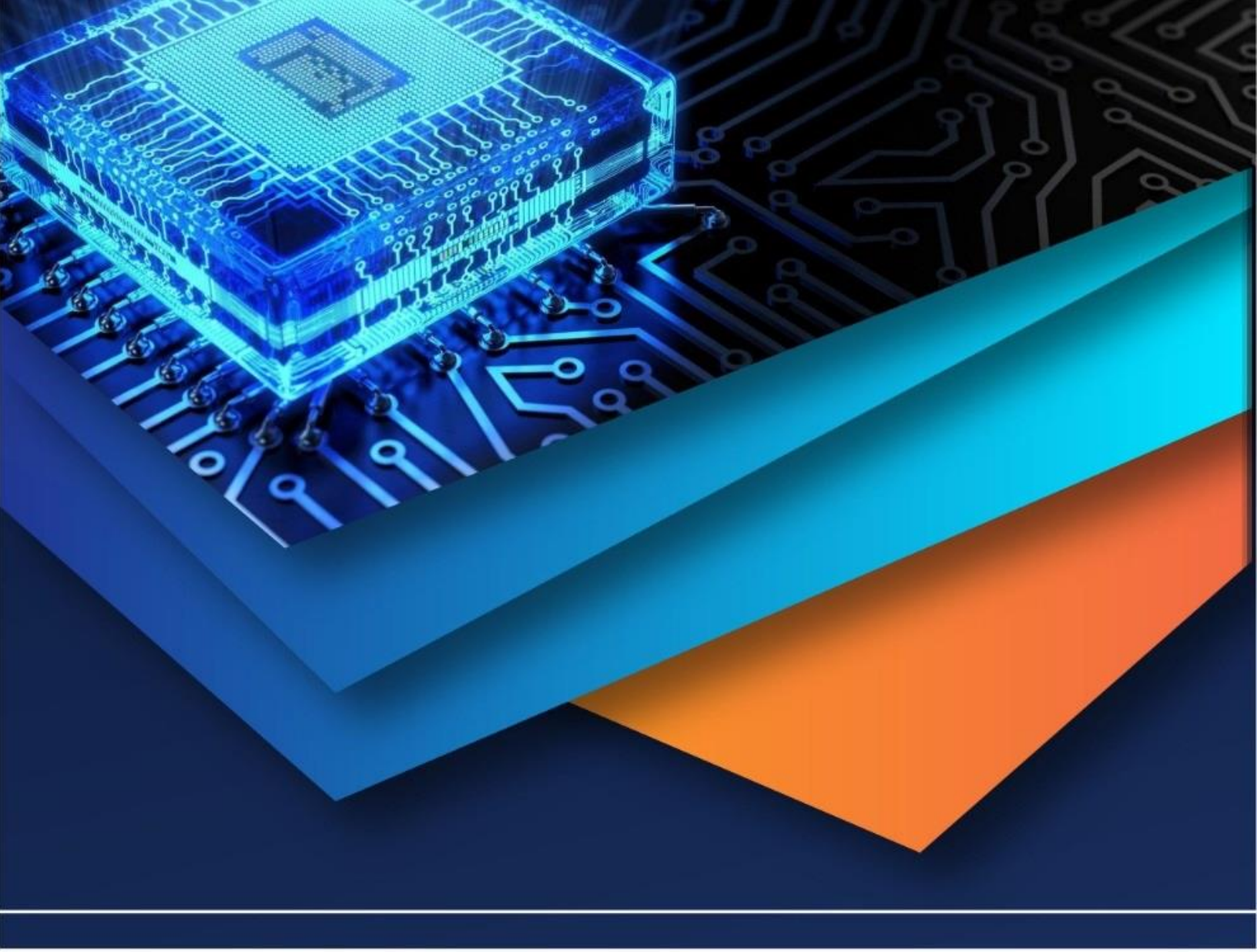


Plate-16 -Fibers as crystal getting arranged in a particular fan like fashion and growing. (3000X)

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