

ELECTRON MICROSCOPICAL OBSERVATION OF CROWN-OF-THORNS' *Acanthaster planci* INTEGUMENT, INTERNAL TISSUE AND COELOMIC FLUID

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The majestic coral reefs of Malaysia are facing a destructive detrimental situation orchestrated by crown-of-thorns (COT). Crown-of-thorns, the second largest starfish endemic is economically non-viable. At present, the only recorded prompt management towards these endemic was to collect and buried on-shore. This worldwide effort is a non-problem solving as it lacks of out-of-the-box-thinking. Effort should be made to generate wealth from this wasted biomass. We believe that being Echinoderms, COT should contain a wide range of therapeutic biocompounds from its biomass such as glycosaminoglycans that is essential in wound healing. As a prelude to that, a pilot detailed histological and electron microscopical observation of the invertebrate was conducted (LM, FEI FESEM and Philips CM-12 TEM). Qualitative analysis or EDX was also conducted to quantify any periodic table element distribution. SEM revealed the integument as woven bone-like structure.

Keywords: Microscopical, Crown-of-thorn, *Acanthaster planci*

INTRODUCTION

Crown-of-thorns (COT) *Acanthaster planci* are large marine starfish that belongs to the phylum Echinoderm. This is a radial venomous marine invertebrate that can grow up to 1 meter in diameter. The average size is only about 25-35cm. Morphologically, the adults COT have 16-18 protrusion arms with dorsally placed sharp poisonous spines. These COTs feed mainly on the tubular coral species, *Acropora* specifically and coral polyps. This feeding habit is detrimental as these invertebrates can eat corals up to their actual body size each day and causing a rapid decline in coral reef ecosystem health [1]. Being the second largest starfish in the world, COT has been incapacitating the coral reefs in Malaysian coastal for years. The only putative way of removing these predators are by buried the collected COT within onshore sand bed [2]. This mode of COT removal although may not be the best of practice for the time being, seems to be the standard procedure SOPD applied worldwide. The invasion of these venomous spiny creatures was so apparent that it requires immediate actions by Marine Park Malaysia annually at popular sites of islands in Malaysia. These involve hundreds of divers who were

volunteers to expel manually these echinoderms from continually devouring the precious coral reefs [3]. Up to date, there is still a scientific lacunae on research of characterizing local *Acanthaster planci* using high resolution microscopy technology to further understand and elaborate different parts of this local echinoderm. In tandem to this, we would like to illustrate an investigative morphological works of various anatomical parts of the COT integrated with energy dispersive X-ray (EDX) analysis. This effort preludes the possible usage of the COT biomass as a waste-to-wealth alternative source of interest in medical and nutraceutical purposes.

MATERIALS & METHODS

Samples Collection

Fresh sample of COT were harvested from Perhentian Island under the supervision of the Marine Park Malaysia Department. The invertebrates were rinsed and their spines dissected out using scalpel and forceps given intention to its highly venomous potential. The body was then separated from the potential arms. The integumental wall and internal tissue were dissected from both body and arms, and coelomic fluid collected via syringe or pasteur pipette from both inner part of body and arms.

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Samples Processing

This study utilized Motic BA210 Biological Upright Light Microscope equipped with EF-N Plan Objective, FESEM JSM-6701F incorporated with energy dispersive X-ray at secondary electron, 6-11 mm working distance and TEM Philips CM-12 with negative stained carbon coated mesh (200) grid in TEM observation of coelomic fluid.

RESULTS & DISCUSSION

Digital photomicrograph of the dorsal and ventral surface of COT. Fig. 1(a) demonstrated the aboral (dorsal) surface while the ventral body [Fig. 1(b)] situates the centrally mouth orifice.

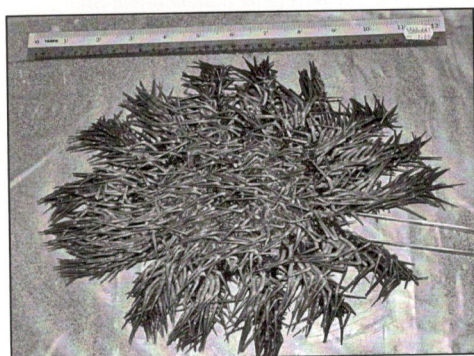


Fig. 1(a). The aboral surface. 26.5 cm diameter.

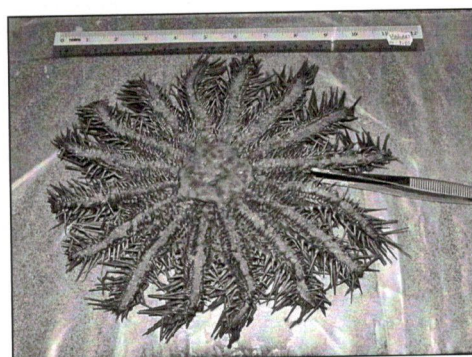


Fig. 1(b). The oral (ventral) surface



Fig. 2(a). Longitudinal view of the integumental wall revealing densely packed connective tissue fibers especially at the peripheral to its outer lining. However this organization become less dense as the fibers move inward. H & E stain. Low magnification with EF-N Plan objective.

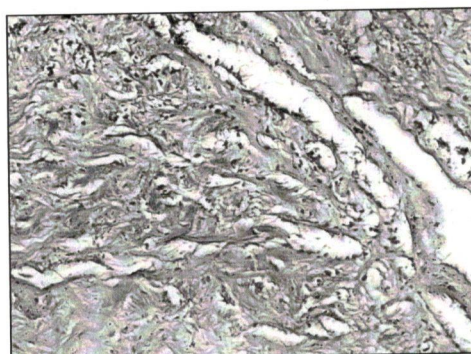


Fig. 2(b). Higher magnification of integumental wall shown the associations of various of collagenous fibers closely adherence to a numerous number of fibroblast-like cells. H & E stain. Low magnification with EF-N Plan objective.

Light microscope observation of both the integumental wall and internal tissue revealed a very thick and dense collagen threads presence as shown in Fig. 2 and Fig. 3. If extracted, these collagenous threads can be an alternative to the various collagen products now being highly commercialized and derived from fish, cattle and bovine source. The alternative is a considerable especially in the halal world and with threats from zoonosis. Fig. 4 revealed the coelomic fluid under light microscope viewing. The overall optical resolution is not giving clear evidence that this is cellular structure. More probing will be conducted of serial sectioning under laser confocal.

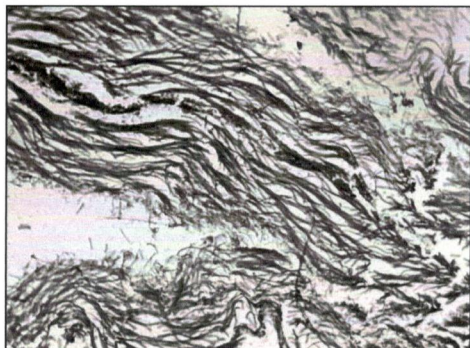


Fig.3(a). Transverse view of internal tissue revealing a thicker bundles of collagenous fibers but less dense in arrangement. H & E stain. Medium magnification with EF-N Plan objective.



Fig. 3(b). Higher magnification of Figure 3(a) revealing longitudinal view of the internal tissue. Thick bundles of spiral wavy arrangement of connective tissue fibers were well defined. H & E stain. High magnification with EF-N Plan objective

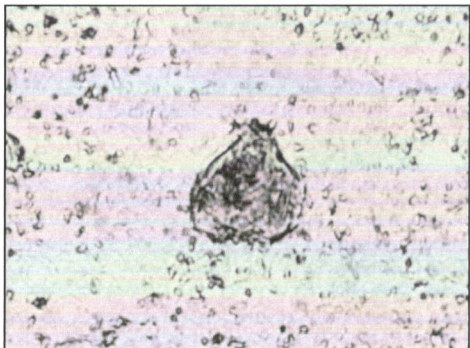


Fig. 4(a). Upright research graded Motic light microscope micrograph revealing union-shape indentation within the coelomic fluid extract. H & E stain. 400x magnification.

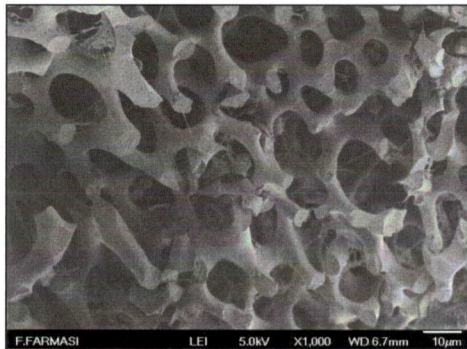


Fig. 5(a). Photomicrograph of FESEM shown the structure of the integumental wall revealing homogenous porous mineralized plates. 1,000x magnification

Photomicrograph of integumental wall using FESEM on Fig. 5(a) exhibited the presents of numerous porous mineralized-like structures giving an osteoporotic-like arrangement. Under higher magnification [Fig. 5(b)], the indent surface of the protuberance seems to show a spiral presentation just like the appearance on the cut surface of a tree trunk. Perhaps, taxonomical and forensic speculation of specific ageing of the said starfish can be studied using this arrangement. Micro-diffraction analysis of the integumental wall confirmed its biological structures with detection of 70.65% carbon, 28.00% oxygen and 1.35% sulfur. The internal tissues observed under FESEM revealing presence of fine collagenous threads. EDX analysis in internal tissue

has resulted with 59.93% carbon, 38.43% oxygen and 1.63% sulfur.

It seems that sulfur is ubiquitously presence at both integument and internal tissue. Although this element of periodic table is generally found in every living tissues of most organisms, it forms an integral component of the biological compartment that forms the human skin, hair, nail and cartilage [4]. Sulfur is also an important element that is responsible in the making of chondroitin sulfate, dermatan sulfate and heparin which are different types of glycosaminoglycan, an unbranched polysaccharide responsible in wound healing process [5]. Hence,

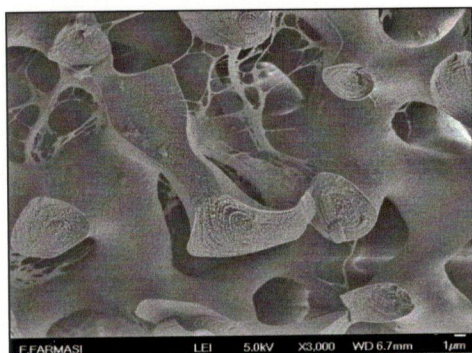


Fig. 5(b). Higher 3,000x magnification at the surface of the protuberance shows stages of different spiral diameters presentation. Seen here fine collagenous threads associated around the porous mineralized structure.

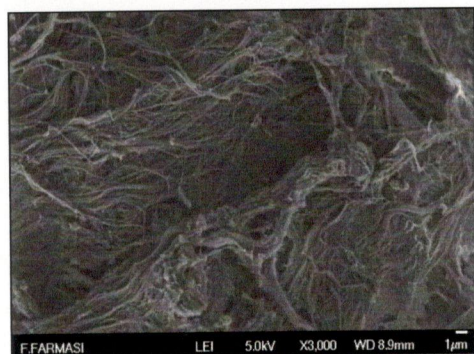


Fig. 6(b). Higher magnification of the fine extrusion linings revealed mesh of very fine collagenous threads. 3,000x magnification.

extracellular collagen matrix and sulfur can be there is much possibilities that the presence of hypothetical tangibilities that the extracts from this integumental wall and internal tissues can be utilised.

There are numerous different orientations of bundles collagen fibers as observed on the TEM photomicrograph in Fig. 7(a). This phenomena maybe due to a tight intertwining of complex nature of the threads. The intertwining phenomena 'creates a suggestion this is a strong bonding and of three-dimensional networking. This three-dimensional network of 64 to 67 nm D-banding characteristic of each individual collagen was well observed [6]. The banding can be clearly observed on the parallel orientation of the said fibers in Fig. 7(a).

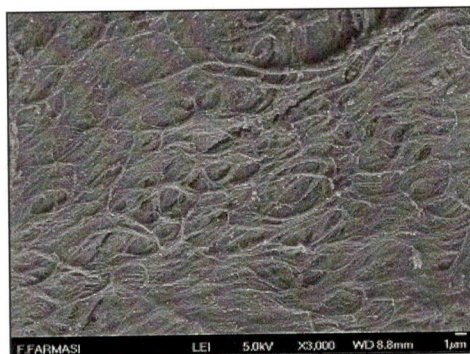


Fig. 6(a). Morphological surface of internal tissue preparation. This low magnification micrograph revealed various mesh work of thread-like structure as in smear layer.. 250x magnification.

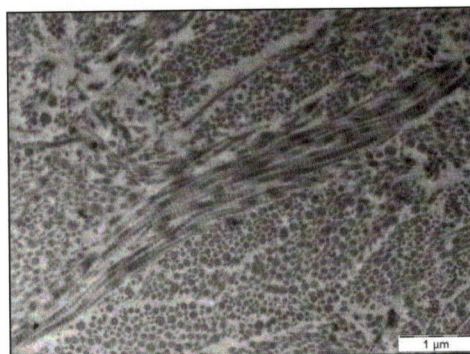


Fig. 7(a). Photomicrograph shown a prominent parallel and cross section orientations of very dense collagen fiber bundle. Clear banding can be observed as the bundle parallel itself. 15,000x magnifications.

Plasma cell (putative) in integument [Fig. 7(b)] and in Fig. 8(a) in internal tissue are inflammatory cells that proliferate during a cascade of infection and are mostly associated with chronic inflammation trilogy. These cells are white blood cells that mediated through a humoral immune response by producing specific antibodies to specific antigens [7]. What is interesting is that this plasma cell seems to be a resident cell within the extracellular matrix of the integumental wall and internal tissue of the COT. Little is known of the actual significance of its presence in a known inflammatory tissue. In other echinoderm, the presence of plasma cell is related to a spherule-cell (morula cell) that is presented with various vesicles within its cytoplasm.

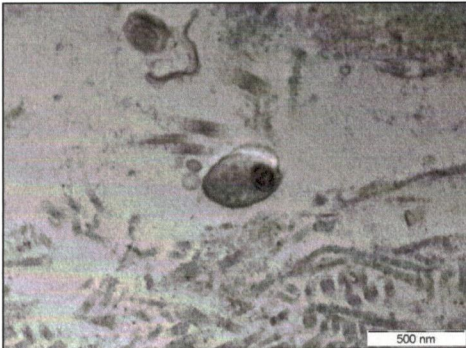


Fig. 7(b). A putative plasma cell in integumental wall with its 2:1 cytoplasmic to nucleus ratio. This cell is associated at its lower part with thick distribution of collagenous fiber. 40,000x magnifications.

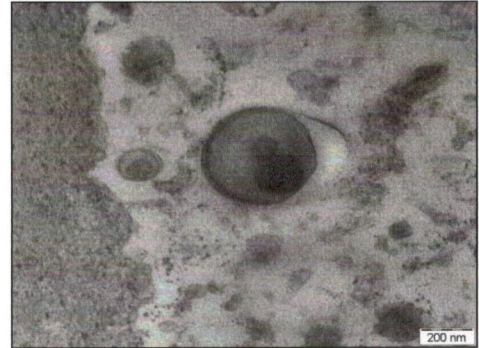


Fig. 8(a). Conventional TEM micrograph of plasma cell closely associated with the chondroid plates. The plasma cell is presented with an eccentrically cartwheel located nucleus. 53,000x magnification.

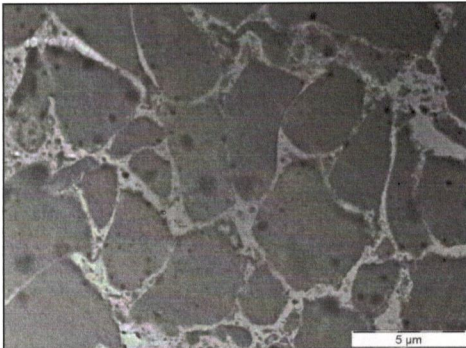


Fig. 8(b). Lower magnification of internal tissue revealed bundles of mineralized chondroid-like structure. This structure is embedded in mineralized specialized-like connective tissue morphology. 4,400x magnifications.

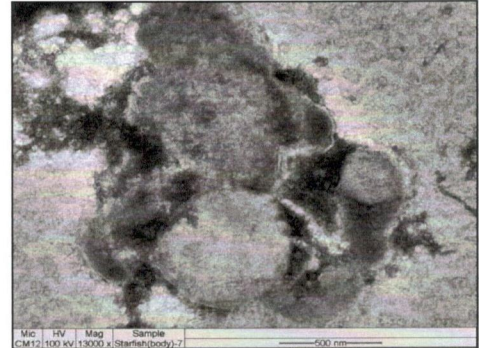


Fig. 9(a). This Philips CM-12 TEM photomicrograph revealed a very thick concentrated electron dense coelomic fluid with multi-putative vesicles and cellular-like structures. The cellular-like structures seem to aggregate around globular-rounded structures with contracted appearance. 13,000x magnification

In Fig. 8(b), plates of chondroid-like structures with bordering collagenous fibers were observed. This chondroid-like plates could be the biomineralization site [8] and although micro-diffraction analysis as in EDX was conducted and calcium has not really detected as in most marine invertebrates, the calcium make up of this chondroid-like structures could be an accumulation of amorphous calcium carbonate matrix [9].

As in most nature's matrix, it is believed that it is a type I collagen that was fabricated and associated to this chondroid structures. In most biological

structures, this will be followed by embedment of calcium crystals and type II collagen that evolved in advance stage in its mineralization [10]. The presence of chondroid plates as biomineralization areas plus the presence of the resident plasma cell and sulfur thus further support its potential in wound healing activities and the biomass as a regeneration model.

The coelomic fluid is believed to contain nutrients required by the starfish. Hemal system in echinoderm for example functions in draining the nutrients from the coelomic fluid into the gonads [11]. The putative vesicles and cellular-like structures seen on both photomicrograph [Fig. 9(a) & 9(b)] of coelomic fluid could be coelomocytes. Coelomocytes

are cells normally found in nearly all echinoderm's coelomic fluid [12]. These intriguing cells are usually involved in recognizing and phagocytosing of foreign materials, including bacteria and also phagocytosing food particles. Aside that, these cells involve in synthesizing pigments and collagen for connective tissue, transporting oxygen and other nutritive materials.

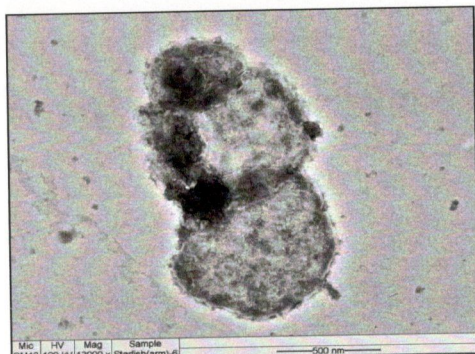


Fig. 9(b). The unstained fluid revealed two cells with clear electron dense cytoplasm with peripherally located nucleus cells on the left corner seems to be presented with three nucleuses. Both cells have clear bilayered plasma membrane. Osmium stained. 13 000x magnification.

CONCLUSION

The characterized cell, extracellular matrix and periodic table element within the various anatomical parts of the COT revealed here suggest the potential of this tangibility in its biomass as a possible alternative source of importance for biotechnological and medical usage. The chondroid-like structures can also be a tool for mineralization study.

ACKNOWLEDGEMENTS

The author would like to acknowledge the help from Mr. Hasbullah Abdul Samad, Mr. Mohd. Shahrizal Yaacob and Ministry of Higher Education for the financial support through Fundamental Research Grant Scheme (FRGS) grant No. 203/PPSK/6171139.

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