The Wound Plug of *Caulerpa sp*., the Giant Coenocytic Alga, Visualized with an In-situ Hummingbird Liquid Cell TEM Holder

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Caulerpa is a genus of coenocytic (single cell, multi-nucleated) tropical marine algae that exhibit the ability to heal after damage caused by morphological or environmental stresses. Wounding of the plant is followed by a coagulated cell wound plug formation. The wound plug is evident by a whiteness in an otherwise healthy green area of the plant in the case of internal wound plugs, or a milky white viscous leakage from the cut area if the wound is external.

In the last 20 years, studies have investigated the mechanism and conformation of biomolecules during the transition from intact plant tissue to wound plug formation of coenocytic algae. In *Caulerpa* specifically the lipase inhibitor caulerpenyne, is biochemically converted into an aldehyde via an esterase seconds after a wounding event. This metabolite conversion leads to a cross linking of proteins in order to quickly close the wound area with minimal loss of cytoplasm [1]. The extent of conversion is dependent on the time the wound plug is allowed to accumulate [2, 3, 4]. These findings have led to the investigation of the use of this process not only as a model system for animal healing but also for the use of caulerpenyne as a coagulant for natural healing.

Prior to the evolution of in-situ TEM holders only fixed, frozen, or replicated material have been used for investigation of these quick forming wounds in the electron microscope. This paper demonstrates a technique to image the wound plug of *Caulerpa* in the purest form possible without any chemical fixation, thus allowing the natural transformation during the biochemical metabolic processes shown in previous research by others. A Hummingbird in-situ liquid cell holder is utilized to study fresh as possible samples. For reference, a comparison of TEM imaging of the wound plug in *Caulerpa sp.*, of traditionally embedded sectioned samples will be shown.

Cultured plants of either *C. prolifera* or *C. racesmosa* were cleaned with fresh sea water, wounded and left to form a wound plug for 30 or 60 seconds and then the wound plugs were extracted with an 18 gauge needle syringe. Only mature healthy stipes of *Caulerpa* were used in the in-situ experiments. While typically most studies let the plug form for several minutes a shorter time was used here in order to keep the plug from becoming too viscous and therefore becoming nearly impossible to withdraw in tiny volumes.

In the 30 second wounded stipes, the presence of organelles that appear to be chloroplasts were found along with trabeculae. Identification of structures within the liquid material is much more complicated due to the lack of contrast compared to using either fixed or even cryo prepared samples so comparisons with fixed material was done. Fixed and ultramicrotomed sections allow for higher contrast but possibly inhibit or change the metabolic processes that are occurring within the plant during wounding. In addition many non-descript and not visibly membranous contents have been seen. It is quite possible the unidentifiable structures are biomolecules that are a result of the quick wounding process.

In summary, this technique shows promise characterizing biological samples such as these coenocytic algae. The Hummingbird liquid cell holder allows visualization of the cell without causing changes to macromoleucles that may occur during fixation.

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References:

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Figure 1.

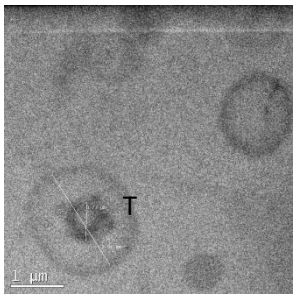
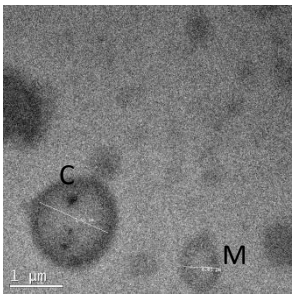


Figure 2.



Figures 1 and 2: Images of from 30 second wound extraction imaged in a Hummingbird in-situ liquid cell TEM holder. The structures in both images appear to be a side view of trabeculae (T) and chloroplasts (C). In figure 2 there appears to be a smaller structure that may possibly be a mitrochondrion (M).

Figure 3.

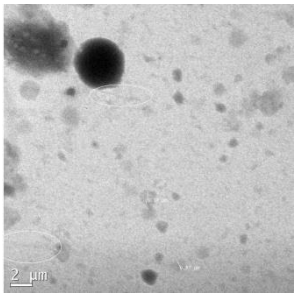


Figure 4.

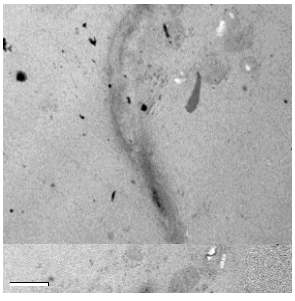


Figure 5.

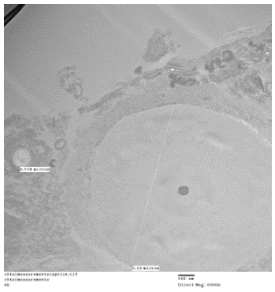


Figure 3: Image from 30 second wound extraction imaged in a Hummingbird in-situ liquid cell TEM holder. The areas circles appear to contain trabeculae in cross length view, the unique to *Caulerpa* rod-like extensions that appears throughout the cell to form a massive scaffolding. The many electron dense particles contained are possibly a result of the metabolic processing from caulerpenyne to aldehydes that occurs during wounding. Figs. 4 and 5 are images from 60 second wounds followed by chemical fixation and ultramicrotoming (unstained and stained respectively) depicting a very large trabeculae.