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The Biomineral-Cell Interface in the Sea Urchin Embryo

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As man-made materials become more similar to the biological structures that inspire them, they increasingly combine nano-sized hard and soft, synthetic and biological components. This creates new challenges for characterization, especially in those materials where water is an integral part of the structure. Cryogenic sample preparation and imaging is often necessary for such specimens. Imaging of cryo-fixed, freeze-fractured samples by cryo-SEM is particularly efficient. However, the propagation of the fracture plane is unpredictable at best, and frequently the fracture surface fails to reveal the interface of interest [1]. This can be a major complicating factor, for example in the analysis of the interaction of cells and the endoskeleton in the sea urchin embryo [2]. Herein, we describe a cryogenic sample preparation workflow for cryo-planing and imaging large areas of frozen-hydrated samples, using whole sea urchin embryos as an example for a hybrid material with large hardness contrast between the organic and biomineralized tissues.

The central innovation of the cryo triple ion gun milling (*CryoTIGM*) method is a custom-built tool based on ion mill slope cutter. Specifically, a Leica TIC3X unit was fitted with a vacuum load lock that allows cryo-transfer of a vitrified sample. Sea urchin embryo suspensions were high pressure-frozen between aluminum planchettes and trimmed using a custom-built cryo-saw. The cryo-saw consists of a liquid nitrogen reservoir, a sample compartment, a diamond blade, and a VCT-docking port (Fig. 1A). Trimming was performed under liquid nitrogen, and samples were then positioned in a sample holder next to a milling mask (Fig. 1B). The sample was transferred to the *CryoTIGM* tool (Fig. 1C), where three broad Ar^+ beams converge at the mask shielding the trimmed sample edge (Fig. 1D). Material above the mask was removed, creating a cross-section in the sample at the level of the mask (Fig. 1E). The ion-milled sample was subsequently freeze-etched and coated with Pt to increase contrast.

For whole, frozen-hydrated sea urchin embryos, we find that ion milling with Ar^+ at an acceleration voltage of 3.0 kV, a current of 1.0 mA/gun, a base temperature of -120^{o}C, and for 2 h results in very smooth cryo-planed area of ~700,000 μm^2 (Fig. 1E). *S*ections clearly revealed cell-endoskeleton interfaces (Fig. 2A). The membranes of the syncytium that envelopes the endoskeleton appear well-defined (Fig. 2B). Numerous organelles are observed within the syncytial compartment, indicating excellent preservation of cellular ultrastructure. These results suggest that *CryoTIGM* is a promising new tool for interfacial studies of hybrid hard/soft materials. Given the large and smooth cryo-planed surface, microanalysis by cryo-SEM-EDS appears particularly promising. In this context, I will discuss recent attempts to identify vesicles that store and/or transport biomineral precursors in the sea urchin embryo.

References:

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[3] The authors acknowledge funding from the NSF (NSF MRI-1229693, DMR-1106208), the Northwestern University Materials Research Center (DMR-1121262).

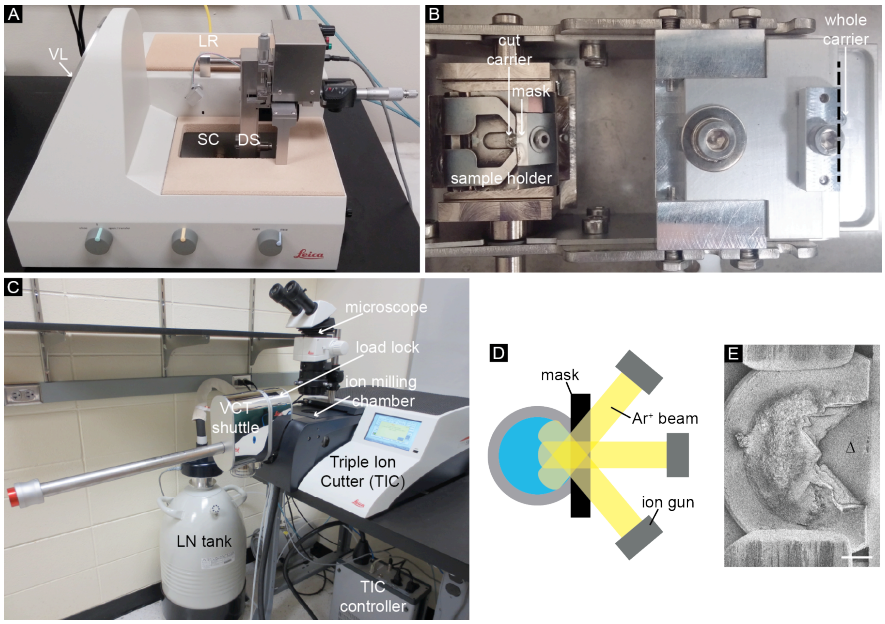

Figure 1. Experimental setup of *CryoTIGM*. (A) The cryo-saw used for trimming sample carriers. DS = diamond saw, LR = LN reservoir, SC = sample compartment, VL = VCT loading dock. (B) Top view of the sample compartment. A sample carrier is trimmed (along the dashed line) and transferred to the sample holder. (C) *CryoTIGM* tool with attached cryo/vacuum-transfer-shuttle. (D) Schematic drawing of *CryoTIGM* milling process. (E) A frozen hydrated sample after milling shows a triangular cryo-planed area of 700,000 µm^2 (Δ). Scale bar represents 500 µm.

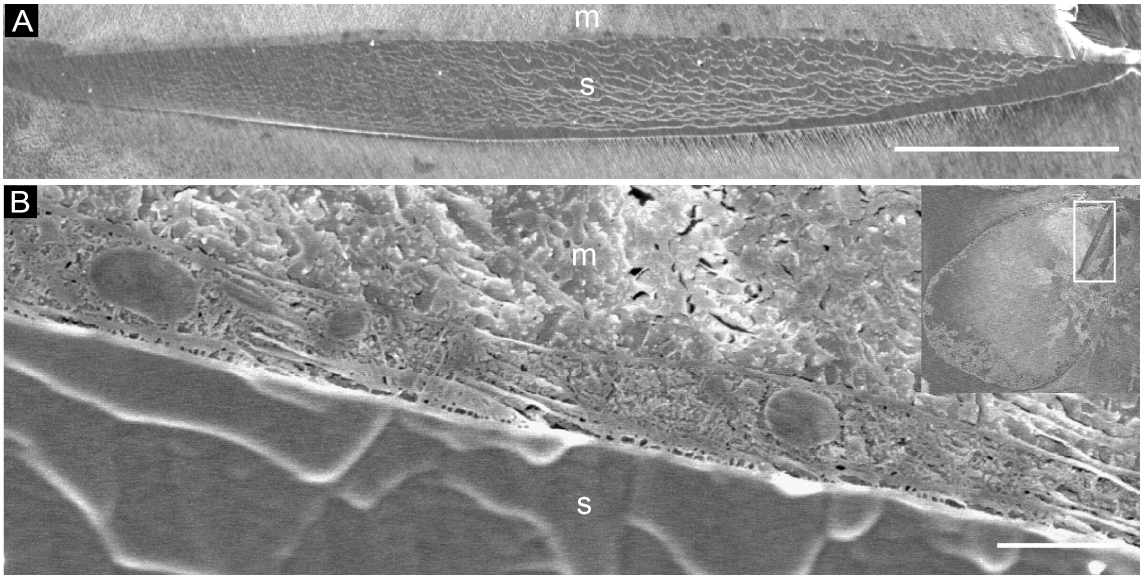

Figure 2. Cryo-SEM of sectioned endoskeleton of sea urchin embryos prepared by *CryoTIGM*. (A) Plane of intercept of the ion beams and the endoskeleton. Milling marks are visible in the embryonic matrix (m) on the two sides of the endoskeleton (s). (B) The cross-section of a syncytium enveloping the endoskeleton. Various organelles are observed within it. The terraces in the mineral region are likely milling artifacts. The inset shows an ion-milled whole embryo, whose endoskeleton is enclosed by a rectangle and shown close-up in panel A. Scale bar represents 10 µm in A and 500 nm in B.