FP7-INFRASTRUCTURES-2012-1

Enabling Science and Technology through European Electron Microscopy

Project Acronym: ESTEEM2

Grant Agreement n°: 312483

Deliverable 6.2

Report on protocols and standards developed in ESTEEM2

Deliverable leader 6 – Max Planck Institute for Solid State Research, Stuttgart

TEM sample preparation of natural layered hybrid materials

The complexity of composite materials, where inorganic and organic components co-exist, demands structural and chemical characterization performed at high spatial resolution. Therefore, transmission electron microscopy (TEM) including a variety of imaging and analytical techniques is an extremely powerful and advanced approach for characterizing biological composite materials. Detailed knowledge and precise understanding of the micro- and nano-structure at the inorganic/organic and inorganic/inorganic contacts and interfaces are of special interest, since they are expected to have a major influence on the mechanical properties of the material and could help to better understand and predict structurally influenced and controlled materials properties in general.

For our study, an abalone shell (*Haliotis*) was used (Fig. 1) [1]. The exact age of the animal investigated is not known. The structure of a typical abalone shell consists of an outer prismatic layer (calcite) and an inner nacreous layer (aragonite). The nacre is an inorganic/organic composite material consisting of polygonal aragonite platelets forming sequential layers that are separated by thin organic layers [2].

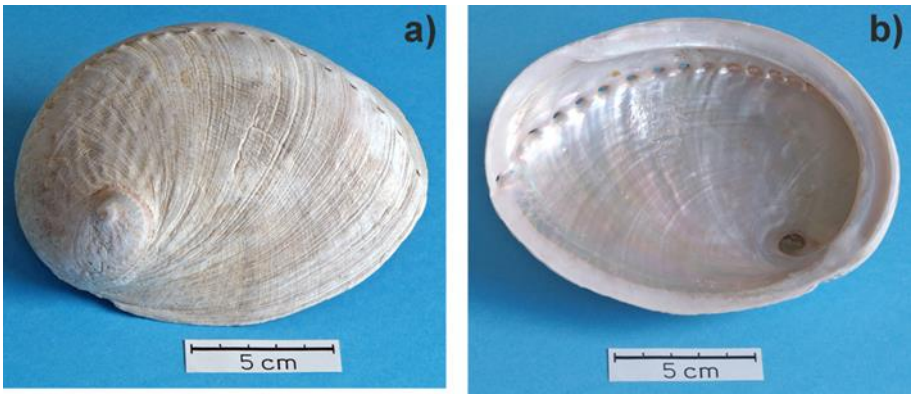

Figure 1: Photographs of abalone shell (*Haliotis*).

TEM sample preparation and the quality of TEM specimens may strongly affect the results of the TEM investigations. This is because during TEM sample preparation significant artifacts can be introduced due to mechanical stresses, elevated temperatures and/or ion-beam induced damaging effects. Therefore special care has to be taken to avoid or at least minimize these artifacts. In order to prepare artifact-free samples from different parts of the abalone shell for imaging and analytical (S)TEM experiments, different TEM specimen preparation techniques, such as classical dimpling and mechanical tripod polishing combined with ion milling with and without specimen cooling, were applied and the results were compared to each other.

The shell of abalone was cut in cross-sectional geometry and the TEM specimens were prepared following the technique developed by Strecker *et al.* [3]. Ar^+ ion-milling was

performed using the Gatan Precision Ion Polishing System (PIPS) at 3 kV and final polishing at 1.5 kV while cooling the specimens with liquid nitrogen (L-N_2) (sample type SP1 in Table 1). In order to study the effect of ion-beam-induced heating on the sensitive biological materials, some samples were ion-milled without cooling (sample type SP2 in Table 1).

Wedge-shaped specimens of shell cross-sections were prepared using an automatic tripod polisher (*Allied Multiprep System*). All samples were additionally Ar^+ ion-beam thinned in a Gatan Precision Ion Polishing System (PIPS) at 3 kV with additional final polishing at 1.5 kV. It is important to note that all the tripod-polished samples were cooled with L-N_2 during ion-milling (sample type SP3 in Table 1).

Table 1. Investigated TEM samples and conditions used for their preparation.

Technique used for TEM sample preparation	*energy used for ion-beam thinning*	*L-N_2 cooling*	*type/name of the sample*
classical dimpling	3 kV, final polishing at 1.5	Yes	SP1
classical dimpling	3 kV, final polishing at 1.5	No	SP2
wedge-shaped polishing	3 kV, final polishing at 1.5	Yes	SP3

Our previous studies of sensitive biological composite materials have shown that there is a great danger of specimen damage when thinning the sample at room temperature by an ion-beam [4]. Experimental and theoretical studies have shown that temperatures of up to several hundred degrees Celsius can be generated at the specimen surface under normal ion-milling conditions [5]. Bright-field STEM images of abalone shell nacre prepared under similar conditions (a) without (sample type SP2 in Table 1) and (b) with (sample type SP1 in Table 1) L-N_2 cooling are shown in Fig. 2. An excessive amount of voids, as an effect of beam-induced heating during ion-milling, were observed when the samples were not cooled (Fig. 2a). Additionally, a higher amount of voids could be observed closer to the edge of the TEM sample. In contrast, the appearance of voids was drastically minimized or completely absent in TEM samples cooled by L-N_2 but otherwise keeping very similar parameters for the ion milling procedure (Fig. 2b).

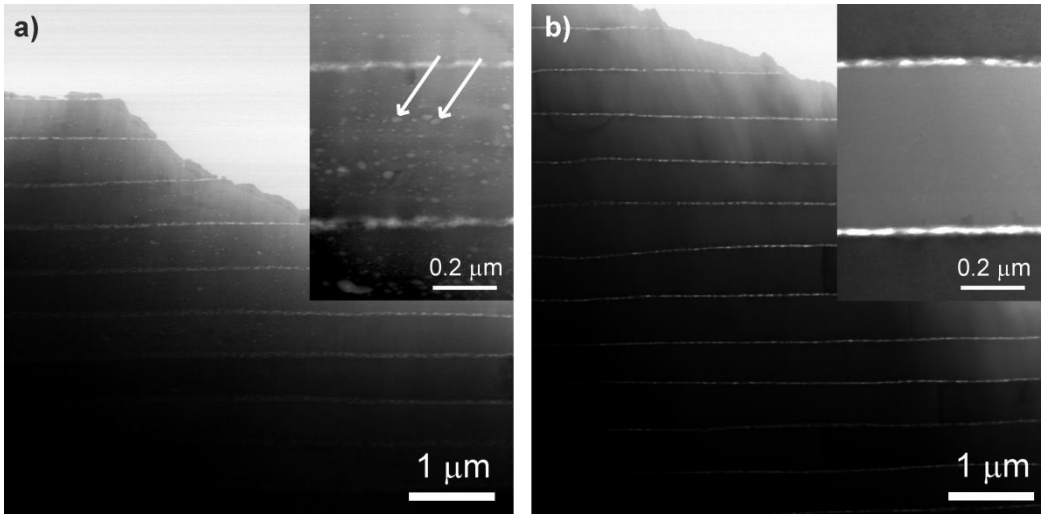

Figure 2: BF-STEM images of nacre consisting of aragonite platelets separated by thin organic layers. (a) Conventional Ar-ion milling introduces voids clearly seen in the aragonite platelets (sample SP2 in Table 1). (b) L-N_2 cooling assisted Ar-ion milling minimizes the formation of structural artifacts like voids (sample SP1 in Table 1).

Considering our above mentioned results it is reasonable to believe, that such voids are not intrinsic to nacreous aragonite platelets but rather an artifact caused by beam-induced heating of the TEM specimen during the thinning process. Therefore, L-N_2-cooling needs to be applied and lower ion-beam energies needs to be used during preparation of TEM samples of these materials in order to avoid structural artifacts and specimen damage.

References:

[1] Srot, V., Wegst, U.G.K., Salzberger, U., Koch, C.T., Hahn, K., Kopold, P., van Aken, P.A., 2013. Microstructure, chemistry, and electronic structure of natural hybrid composites in abalone shell. Micron 48, 54-64.

[2] Mann, S., 2005 (reprint). Biomineralization - Principles and concepts in bioinorganic materials chemistry, first ed. Oxford University Press, New York.

[3] Strecker, A., Salzberger, U., Mayer, J., 1993. Specimen preparation for transmission electron microscopy: reliable method for cross-sections and brittle materials. Praktische Metallographie 30, 482-495.

[4] Srot, V., Bussmann, B., Salzberger, U., Koch, C., van Aken, P.A., 2012. Linking microstructure and nanochemistry in human dental tissues. Microscopy and Microanalysis 18, 509-523.

[5] Viguier, B., Mortensen, A., 2001. Heating of TEM specimens during ion milling. Ultramicroscopy 87, 123-133.