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TEM sample preparation of sensitive hybrid biological materials

Transmission electron microscopy (TEM) investigations are highly dependent on the quality of the examined specimens. Preparation of biological materials demands transformation of specimens from a living, hydrated state to a dry state which could cause significant changes. Human teeth are composed of three hard dental tissues. Enamel, the outside part of the teeth, protects the next underlying layer, which is dentine. The outer layer of dentine in the root area is covered by cementum. Mature enamel consists of up to 96 wt% of inorganic material and the rest is composed of water and organic matter. The concentration of organic material in dentine is considerably higher (~ 20 wt%) compared to enamel which is also the reason for different properties of enamel and dentine. TEM sample preparation of human teeth, especially from DEJ was a very challenging task because of the above mentioned reasons.

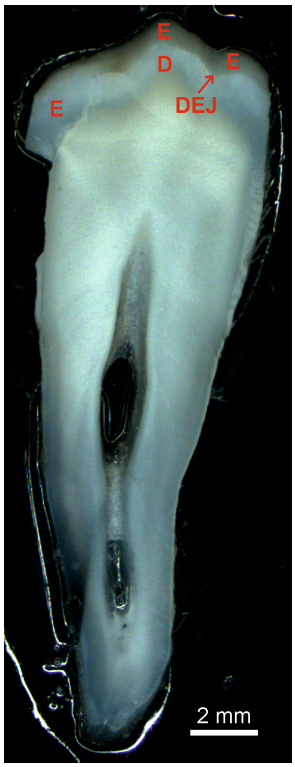

Figure 1: Optical micrograph of a longitudinal section of the permanent human tooth. TEM samples were prepared from several areas in enamel (E) and from dentine-enamel junction (DEJ) regions. E: enamel, D: dentine, DEJ: dentine-enamel junction.

Teeth were longitudinally sectioned and TEM samples were prepared from several positions in enamel (E) and from the dentine-enamel junction (DEJ) (as marked on Figure 1). To avoid potential TEM specimen damage caused by extensive ion-milling, specimens were prepared by the mechanical tripod polishing technique using an automatic tripod polisher (*Allied Multiprep System*). First the samples were cut into slabs of ~ 1.5 mm wide and 2 mm long (Figure 2a) and attached to a pyrex specimen holder using Crystal Bond thermoplastic wax. For sensitive biological materials it is important that the temperature used for attaching the samples is not too high. The specimens were first polished from both sides using diamond-lapping film (DLF) with 15 µm grain-size to provide a planar surface. For further polishing steps 6, 3, 1, 0.5, and 0.1 µm grain-size DLFs were used. A final polishing was performed on a polyurethane cloth using a silica solution with 20 nm particle size (*Allied Colloidal Silica Suspension*) to remove scratches from the polished surface. Between every polishing step the specimens were regularly monitored using the optical microscope. Before polishing the other side, the samples were removed from the pyrex holder by heating the polishing block on a hot plate. In order to polish the second side, specimens were turned upside down and glued again onto the pyrex specimen holder. The specimens were thinned down to a thickness of 200 µm using 15-µm DLF. Afterwards, a wedge angle of 2-3º was introduced. The specimens were polished down to a thickness of 70, 50, 30, and 10 µm by using 15, 6, 3, 1 µm DLFs, respectively. Subsequently, the final polishing was performed with 0.5 µm DLF and 20 nm colloidal silica to eliminate existing surface scratches (Figure 2b). After finishing all the polishing steps, the samples were glued on 3 mm molybdenum (Mo) support half-ring (Figure 2c) and left in air over night to allow the glue to dry. The samples were covered with petri dish to avoid possible mechanical damage. All samples were additionally Ar^+ ion-beam thinned in a Gatan Precision Ion Polishing System (PIPS) (Figure 2d). It is important to mention that all the samples were cooled during ion-milling using liquid nitrogen (L-N_2).

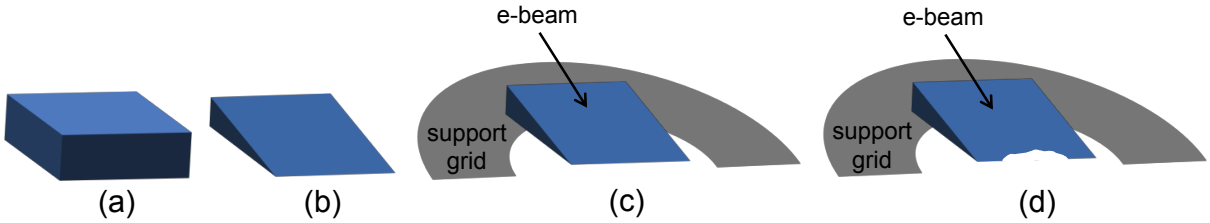

Figure 2: Schematic diagram showing different steps of tripod polishing with subsequent ion-milling.

In Figure 3 bright-field (BF) scanning TEM (STEM) images acquired from enamel (E) (Figure 3a) and from dentine-enamel junction (DEJ) (Figure 3b) from the sample prepared by tripod polishing technique followed by ion-milling (as described above) are shown. Hydroxyapatite (HA) crystals shown in Figure 3a appear to be intact without any noticeable change of the microstructure and chemical composition. In Figure 3b a wide region from DEJ is shown.

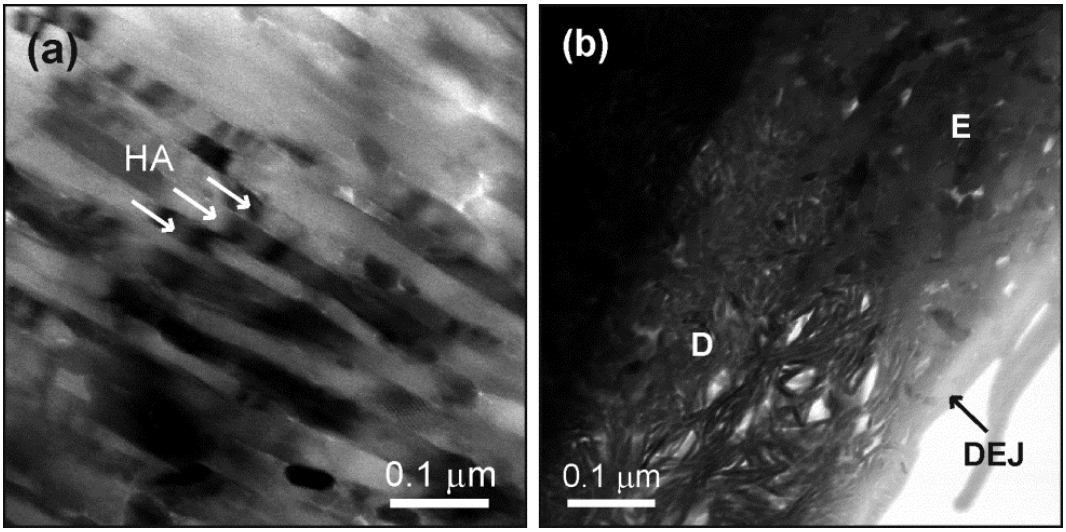

Figure 3: BF-STEM images of (a) enamel and of (b) DEJ region are shown. In both cases the structure appears to be intact, no visible changes could be observed. HA: hydroxyapatite crystals, E: enamel, D: dentine, DEJ: dentine-enamel junction.