

## High resolution ultrastructure imaging of fractures in human dental tissues

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**Abstract** Human dental hard tissues are dentine, cementum, and enamel. These are hydrated mineralised composite tissues with a hierarchical structure and versatile thermo-mechanical properties. The hierarchical structure of dentine and enamel was imaged by transmission electron microscopy (TEM) of samples prepared by focused ion beam (FIB) milling. High resolution TEM was carried out in the vicinity of a crack tip in dentine. An intricate “random weave” pattern of hydroxyapatite crystallites was observed and this provided a possible explanation for toughening of the mineralized dentine tissue at the nano-scale. The results reported here provide the basis for improved understanding of the relationship between the multi-scale nature and the mechanical properties of hierarchically structured biomaterials, and will also be useful for the development of better prosthetic and dental restorative materials.

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Human dental tissues are nano-structured, hierarchical materials that combine organic and mineral phases in an intricate way to obtain a remarkable combination of mechanical strength, thermal endurance, wear resistance, and chemical stability. The multi-level composite structure is illustrated in Fig. 1. The mineral hydroxyapatite (HAp) crystallites glued together by organic matter are the main structural feature both in dentine and enamel at the nano-scale, as well as the dentinal tubules. The stacking arrangements of enamel “keyhole”-shaped rods are, however, distinct at the micro-scale. Knowledge of these ultrastructural features (in the enamel, dentine, and their interface, the dentine-enamel junction (DEJ)) is important for understanding the natural properties of the tissues and the mechanisms by which these load-bearing tissues contribute to the life-long success of human teeth. Such understanding should help identifying the factors that affect the performance of dental tissues, such as disease, treatment, environment, and thermal exposure. It should be also advantageous for the development of biomimetic materials and their artificial replacements, e.g., dental restorations.<sup>1</sup>

Interesting results pertaining to the structural and thermo-mechanical properties of human dental tissues have been obtained by a range of techniques, with particular reliance on synchrotron X-ray scattering and imaging techniques, as well as microscopy techniques.<sup>2-7</sup> Further studies of

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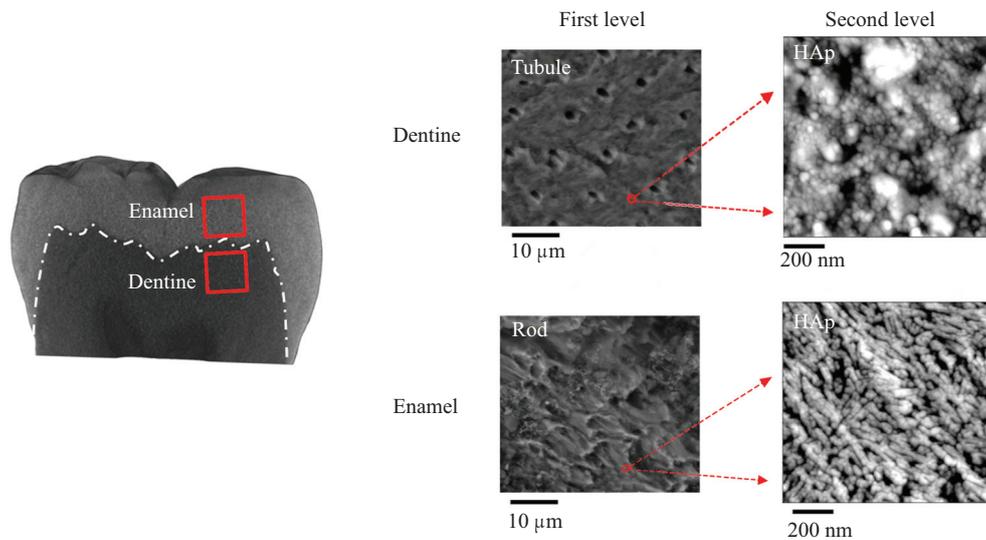


Fig. 1. Multiple microscopic observations of hierarchical structure of human dental tissues.<sup>1</sup>

dentine, enamel, and DEJ should be carried out at ultra-high spatial resolutions in order to reveal details on the deformation and load transfer mechanisms operating in the human tooth. Direct visualization of the internal architecture of these materials is a challenging task that can not be readily accomplished by any single conventional microscopy method, and requires a combination of techniques. Here we present some recent results that rely on combining spatially selective (at the micro-scale) focused ion beam (FIB) milling of regions of human teeth to extract lamellae for transmission electron microscopy (TEM) nano-scale imaging.

FIB instruments have revolutionized the way in which biological samples can be investigated, by providing access to judiciously located extraction of high quality TEM samples. The “lift-out” was carried out using LYRA3 XM (Tescan-UK Ltd.) instrument in the multi-beam laboratory for engineering microscopy (MBLEM) located in the Solid Mechanics and Materials Engineering group, Department of Engineering Science, University of Oxford.<sup>8</sup> The region of interest that incorporated the DEJ was first examined using FIB scanning electron microscope (FIB-SEM) microscopy. FIB-deposited platinum was essential as protection for the sample surfaces during FIB milling. Following a series of rough milling and fine surface polishing steps, a lamella (100–200 nm thick) is obtained. Finally, a nano-manipulator needle is used to attach, lift-out and re-attach the lamella onto a TEM copper grid, to be imaged under the TEM.

This microscopy modality enables imaging the internal sample structure at significantly higher resolution than, for example, SEM. A tightly focused beam of electrons is transmitted through the ultra-thin specimen, and bright-field or dark-field detection is used to form a raster image.<sup>9</sup> There are two primary reasons for the resolution improvement. Firstly, the small sample thickness avoids the “spreading out” phenomenon that affects the electrons entering the sample surface and resulting in a typical “mushroom” pattern formed below the surface in the SEM mode. Secondly, the electron beam energies used in TEM are typically higher than in SEM, leading to shorter de Broglie wavelengths. In order to investigate the arrangement of HAp crystallites around the crack in dentine and enamel at the nano-structural level, TEM imaging was applied

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