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Atomic Resolution STEM Imaging of Human Enamel Crystallites and Characterization of its Localized Impurities

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The hardest tissue in our body, enamel, consists of about 96 wt% mineral and 4 wt% organic molecules and water [1]. Its structure consists of hexagonal-shaped crystallites, bundled together in a complex dense 3D weave. The mineral is nominally hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$; HAp), but is known to contain low concentrations of other ions, such as magnesium, fluorine, and carbonate [2]. The complex chemistry, together with the well-organized structure, play a key role in how enamel is affected by exposure to acid in the oral cavity, which can eventually lead to caries formation. Previous research suggested that dissolution of crystallites at the nanoscale is rather inhomogeneous, where either the periphery of enamel crystallites dissolves preferably during acid attack [2,3], or the center [3]. The presence and identity of these impurity ions has been shown to have a drastic effect on the solubility of HAp in both rodent [4] and human enamel [2]. Thus, knowledge of the ion distribution on the length scale of individual crystallites is crucial to understanding processes of caries formation and progression, and may lead to novel treatment methods. However, it is a challenge to study crystallites at these length scales using conventional electron microscopy due to interference of severe electron beam damage [2,6].

Atom Probe Tomography (APT) showed evidence for the presence of a Mg-substituted amorphous calcium phosphate in between the crystallites in murine enamel [4]. When treated with fluoride, these intergranular regions displayed a co-localization of F and Mg. Similar regions containing Mg and Na were also observed in human enamel [5]. Indeed, when we fluoridated human enamel, APT analysis showed co-localization of Mg (0.57 at%), F (0.74 at%) and Na (2.48 at%) in the interphase region (Fig. 1a, c, d). However, the ion distribution inside the crystallite was more complicated than previously reported. The very center of the crystallite appeared to contain negligible amounts of Mg and F, but a relatively high concentration of Na (1.85 at% according to a 1D concentration profile (Fig. 1a, b). On either side of this central feature, a region rich in both Mg (1.10 at %) and F (0.43 at%) was detected.

The chemical distribution in the APT reconstructions suggest the presence of a linear feature in the center of the crystallite, between the two Mg-rich regions. This is likely consistent with previous observations of a sub-nm sized central dark line (CDL) in HRTEM and STEM-HAADF imaging [4,7]. STEM-HAADF images from focused ion beam (FIB) sections of human enamel (Fig. 2a-b) show regions of lower scattering contrast in the center of the crystallite, along the CDL, similar in size and geometry to the Mg/F-rich regions inside the crystallite observed in APT (Fig. 2b). This can be explained by the substitution of a lower atomic mass element (Mg or Na) for Ca. There is also a lower contrast region along the edge of the crystallite, consistent with the elevated levels of Mg and Na present in the interphase between crystallites. To observe this at higher spatial resolution and reduce beam damage, we imaged our enamel sample under cryogenic conditions in an aberration-corrected STEM (Titan Themis). The cross-correlation of many low exposure images (dwell time 2 μs; 0.17 nA beam current) demonstrated at atomic resolution that the CDL was visible near the [010] zone-axis (Fig 2c), but not further tilted from this axis (Fig. 2d). With an EELS Gatan GIF Quantum set up for spectroscopy at this resolution we always severely

damaged the enamel (not shown), however, we also performed EELS with a Gatan K2 detector, improving frame rates by direct electron counting at high energy resolution. Without direct damage observation, it was possible to map simultaneously edges associated with P, Ca, O, Mg and Na (Fig. 2f-h). Thus, for the first time we image a section of human enamel prepared by FIB at atomic resolution, and quantify chemical impurity content within the crystallites.

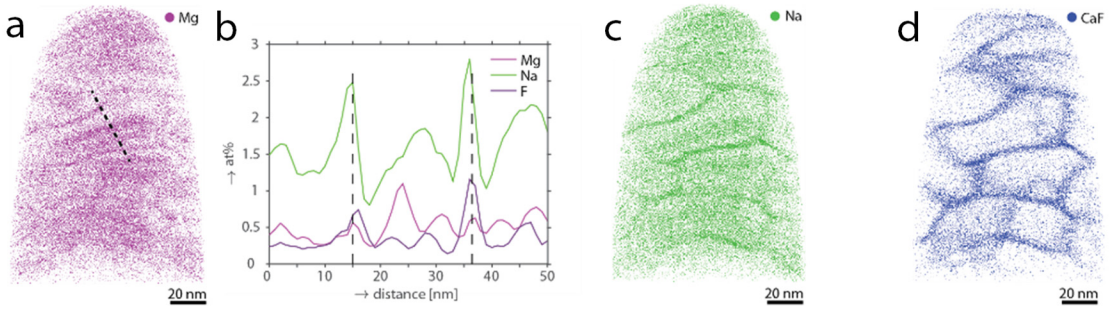

Figure 1. APT reconstructions of human enamel exposed to 250 mM aqueous NaF at 37°C for 24 hours with b) corresponding 1D concentration profiles taken along the crystallite (black dotted line) in a). Only Mg^{2+} ions are visible in a), c) shows Na^{+} ions, and d) CaF^{+} ions.

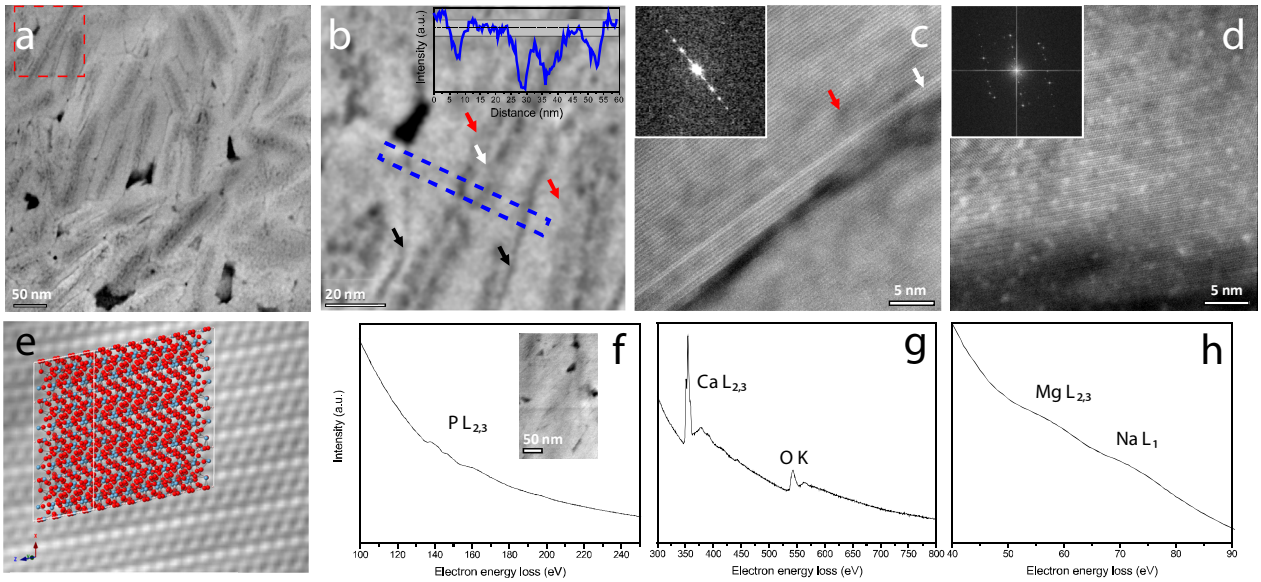

Figure 2. a) STEM-HAADF image of crystallites in human enamel. b) Magnification of red dashed box in a). The blue dashed box indicates the area where the intensity linescan (inset) was taken from (left to right). c,d) aberration-corrected STEM images with FFT (inset). c) is a crystallite near the [010] zone-axis. e) FFT filtered zoom-in of d), with overlay simulated HAp crystal structure along the [02-1] zone-axis. f-h) shows EELS spectra collected with the K2 camera integrated over the area in f), inset. White, red & black arrows in b,c) denote CDL, individual crystallites, and intergranular regions, respectively.

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