3D Nanostructuring and Nanoanalysis of Human Enamel and Dentin Using Focused Ion Beams

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ABSTRACT

This study particularly focuses on three-dimensional nanoanalysis of human dentin and enamel samples, using FIB nanostructuring methods such as FIB tomography and preparation of 3D TEM samples. Features within the human dentin and enamel with micro and nano sized were revealed with high definition and accuracy. While the micro sized features reconstructed in three dimentions by means of stacking 2D SE images which are aquired by slice and view application of FIB/SEM platform, a novel pin-like TEM sample was prepared with FIB to investigate the nano sized features within the dentin and enamel. The complimentary analysis helped to reveal the microstructure and morphology of human dentin and enamel in three dimensions in detail.

Keywords: FIB/SEM, TEM, tomography, dentin, enamel, 3D analysis, dentistry

1 INTRODUCTION

Dental hard tissue comprises a combination of enamel and dentin, both of which have different compositions and structures. Such materials have both micro- and nanometer sized channels, pores and features within the structure. One of the well-known ways of revealing those features is X-ray tomography which is not sufficient due to resolution limits. For having a precise and reliable characterization of small features, 3D electron microscopy is a convenient application [1].

Dentin is a hydrated hard tissue that covers the majority of human teeth by both weight and volume [2] The tissue serves as an elastic foundation for the hard, outermost enamel, and as a protective enclosure for the central pulp. Dentin consists of microscopic channels, called dentinal tubules, which radiate outward through the dentin from the pulp to the exterior cementum or enamel border. These tubules contain fluid and cellular structures [3]. As a result, dentin has a degree of permeability, which can increase the sensation of pain and the rate of tooth decay. Dentin is traversed by a network of tubules that are oriented radially outward from the central pulp towards the dentin–enamel junction [2]. On the other hand, type-I collagen forms a fibrous three dimensional network structure which build up the dentin matrix. Compared to bone, the collagen matrix in dentin is more interwoven with numerous crossing of fibrils. The layers of a human tooth are demonstrated in Fig. 1.

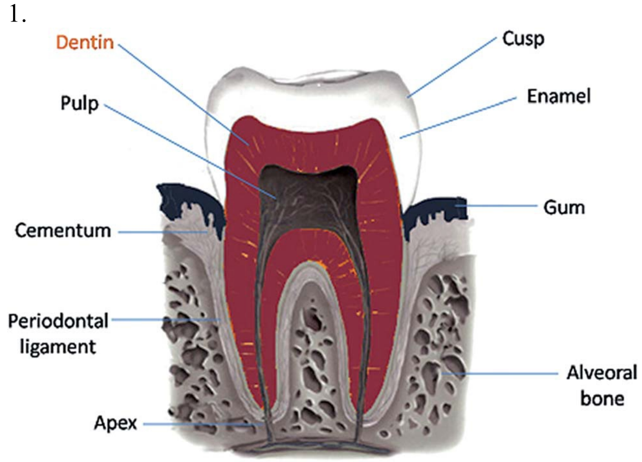

Figure 1: The scheme of a human tooth showing the individual layers.

Enamel is the hardest biological substance in the human body and is a composite material consisting of both a mineral and an organic phase. The mineral phase predominates (95–96 wt.%) and consists primarily of calcium phosphate salts in the form of large hexagonal hydroxyapatite crystals that are both carbonated and defective. Sets of similarly orientated crystals form rod-like structures called enamel prisms, 3–6μm in cross-sectional diameter. Prisms are separated from each other by a thin organic prism sheath and by interprismatic enamel. The protein/organic matrix comprises approximately 1 wt.% of the enamel, and the remaining approximately 3 wt.% is contributed by water.

As the demand for providing information at nano and subnano scale especially in the field of "life sciences" increases, the scientists are in search of employing cutting-edge analysis technologies and developing novel methodologies over advanced characterization tools. Focused Ion Beam (FIB) technologies include the most versatile methods enabling nanoscale prototyping and structuring of materials besides simultaneous site-specific analysis. In this study FIB structuring applications were used in combination to Scanning Electron Microscopy (SEM) imaging in order to gain three-dimensional information on the enamel and dentin structures, providing resolution ranging from micrometer down to nanometer scale. The procedure was performed in two steps. First, for revealing the distiribution of tubules inside dentin 2D slice-series images were acquired for a 3D volumal information of the structure. This procedure was performed once again for enamel. Moreover, FIB/SEM platform was used to prepare a pin-like TEM sample which avoides projection problems during TEM imaging and tomography tilt series.

In addition, previously, it has been reported in the literature that FIB/SEM dual-beam platforms have been extensively used on dental structures, such as dentin or enamel, for either specimen preparation for TEM [4-7] or 3D micro structural analysis using slice & view methods [8]. However there has been no complimentary study yet, that combines the investigation on the micron-sized features of dentin (e.g. tubules) and with the nanofeatures (e.g. collagen fibrils) and comparing it with enamel using both the nanostructuring facilities of dual-beam platforms and the TEM analysis on the FIB-processed 3D specimens.

2 EXPERIMENTAL

Prior to characterization of human dentin and enamel, a human tooth was mechanically cross-sectioned with a die for a better view of layers within the structure. As shown in Fig. 2, the tooth was channel treated and had fillings in the channels nearby dentin layer. A metal thin film was sputter-coated on the specimen prior to the investigations in order to avoid the charging effects caused by both electrons and ions. An area on the sample was chosen as region of interest to do the FIB investigation. For the FIB slice & view series and the following 3D construction a volume of approximately 18 x 17 x 16 mm was set. Within this region, via ion milling, 332 slides were cut with a 50 nm step, using 1 nA ion current at 30 keV ion energy. The secondary electron (SE) images of the slice & view series was acquired at 2500x magnification simultaneously from the cross-sectioned regions. This work has been carried out at a dual-beam platform from JEOL Company: JIB 4601F MultiBeam System having a FEG SEM column and a gallium-ion column, equipped with gas injection systems and a micromanipulator.

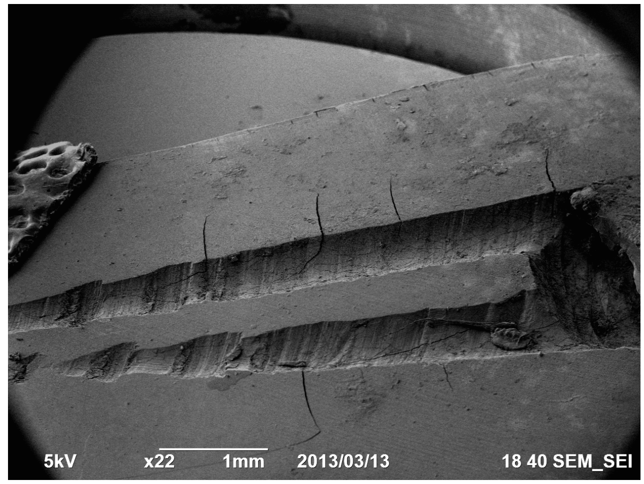

Figure 2: SE image showing the human tooth and the dentin layer around the channels. The selected areawith the yellow square shows the region where the FIB sectioning was done. The FIB tomography carried out on the region has dimensions of x = 18 µm, y = 17 µm and z = 16 µm.

The next step was using TEM in order to track the nano-sized paticles. However, conventional sample preparation has projection problems for doing electron tomography. To overcome this problem a novel pin-like TEM sample was prepared. This was performed using in-situ lift-out technique by the help of a micromanipulater. In order to prevent gallium implantation, initially two protective platinum layers were deposited on top of the region of interest before trenching with annular patterns. The free sections were then mounted on a TEM grid and thinned further by ion milling until thin samples (around 100 nm) were formed. In the final stage, TEM samples were polished, using rather low ion currents for the removal of gallium which might have been implanted on the specimen surface during the milling process. The result was a pin-like transparent TEM samples. Providing detailed morphological and information in three-dimensions was achieved due to the uniformity of pin-form geometry. For this work a JEOL JIB 4601F MultiBeam platform was used. The SE images corresponding to the stages of pin-like TEM specimen preparation are given by Fig. 3.

The preparation steps can be summarized as following: (a) the deposition of protective electron beam assisted platinum layer (with a diameter of 5 mm) onto the region of interest; (b) the deposition of protective ion beam assisted platinum layer (with a diameter of 5 mm) onto the top of electron beam assisted deposit; (c) ion milling of pillar shape sections using intermediate ion beam currents (e.g. 0.1–1 nA) within annular patterns (the yellow circle corresponds to the annular pattern); (d) the lift-out of the section from the dentin structure using a micromanipulator; (e) mounting the pillar to the TEM grid using micromanipulator and platinum deposition; (f) final

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thinning of the sample using low ion currents (e.g. 10–100 pA).

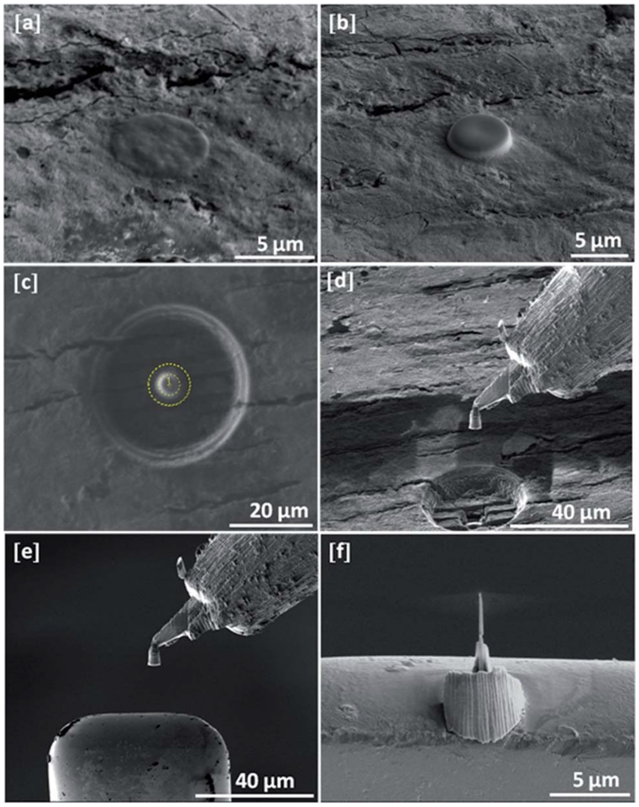

Figure 3: The steps for preparation of pin-like TEM sample using the dual-beam instruments: (a) deposition of electron beam assisted Pt layer, (b) deposition of ion beam assisted Pt layer, (c) ion beam milling via annular patterns, (d) lift-out of the pre-section, (e) mounting of the pre-section onto the grid; (f) final thinning and polishing.

3 RESULTS AND DISCUSSION

This work provides a thorough characterization of human dentin and enamel in 3D. For this different high resolution microscopy techniqyes were used to reveal the interior features of dentin and enamel with high definition and accuracy. In this study we have investigated two particular part of human tooth: *(i)* **Dentin;** which contains micro sized tublues and nanosized collagen fibers; *(ii)* **Enamel;** which contains prisms as interior feature.

Concerning the dentin layer, the objective was to examin the the tubule distribution, alignment and orientation within the structure which is available by slice and view technique in dual beam platform. The mean number of tubules inside a 342 mm2 surface for 332 slides is about 13. This gives a number for the tubular density of approximately 33 000 consistent, with previous 2D studies [9] However 3D observation makes it much easier to examine the tubule number densities. The different images of dentinal tubules distribution are shown in Fig. 4.

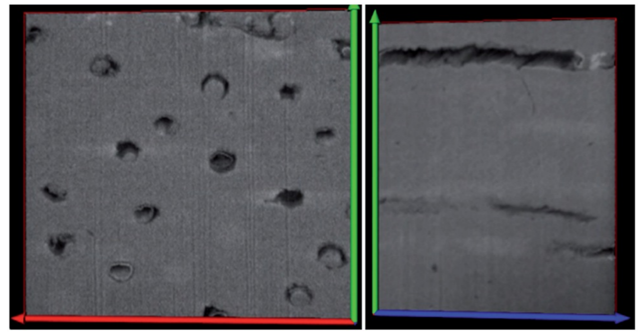

Figure 4: SE images showing the distribution and alignment of the tubules for 3 axes: red arrow correspond to x axis, whereas blue arrow represent the y-axis and green arrow the z axis. The image on the left hand side shows the cross-sections of the tubules along the y axis and the image on the right hand side shows the tubules along the x axis.

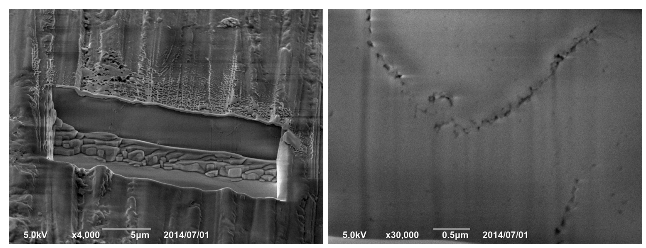

Figure 5: SE images showing the FIB cross-section of the enamel layer: the cross-cut on the surface and magnified SE image for observing the prisms.

In the next step pin-like thin sample was investigated in an FEI TF30 TEM system and micrographs were acquired at 300 keV electron energy. The 3D electron tomography of dentin at the nanoscale revealed the 3D network of collagen fibrils whitin the dentin. The corresponding TEM images are given in Fig. 6.

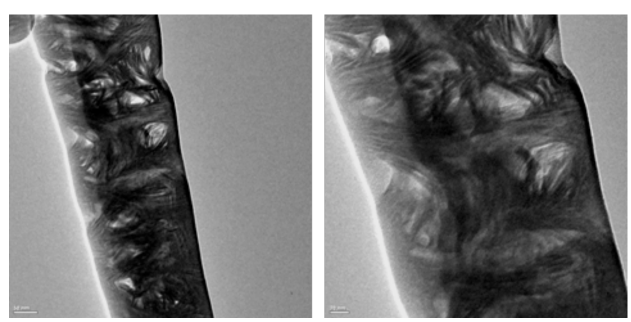

Figure 6: TEM images showing the 3D distribution of collagen fibrils within the human dentin. The micrographs show the nano features within the dentin structure.

Transparent and normal dentin intertubular mineral crystallites appeared needle-like in morphology when observed on edge, similar to previous observations of dentin by other groups [10-11].

On the other hand, the TEM micrographs acquired from the enamel structures which were also prepared as pin-like samples showed a completely different morphology when compared to dentin structures. The crystalline structure was revealed by TEM while no significant porosity in nano or meso-scale was observed.

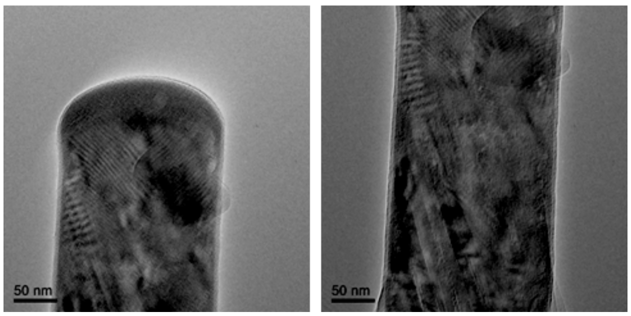

Figure 7: TEM images showing the crystalline structure of human dentin.

4 CONCLUSION

In this work, complimentary electron microscopy analysis including Focused Ion Beam (FIB), Scanning Electron Microscopy (SEM) and high resolution Transmission Electron Microscopy (TEM) were used for observing micro and nano-details within human tooth for dentin and enamel layers. Dual-beam instruments allowed for preparing pin-like uniform and representative 3D TEM specimens using ion-milling and deposition based advanced nanostructuring techniques. This was followed by acquiring related secondary electron (SE) images monitoring micro-sized dentinal tubules in dentin and prisms in enamel tissues. As a complimentary analysis FIB-structured pin-like samples were investigated at the TEM for observing the nanometer sized collagen fibrils that form dentinal tissues and the crystallinity in the enamel strcutures. Consequently, this study enabled for revealing the microstructure and morphology of human dentin and enamel layers in three dimensions for both their micro and nano constituents.

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