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Characterization of Stannous Fluoride Uptake in Human Dentine by Super-X XEDS and Dual-EELS analysis

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Stannous fluoride (SnF_2) is a common additive to dental products and has been shown to reduce the dental hyper-sensitivity in patients. In order to elucidate and better understand the permeability and mass transport mechanisms, analytical electron microscopy (AEM) characterization was performed on human dentin exposed to SnF_2 [1]. In particular, techniques such as S/TEM-HAADF imaging, Super-X XEDS and dual-EELS have been used to investigate the ultrastructure and chemistry of the inner dentine tubule surface. Results on the characterization of the Sn-reacted product on the inner surface of dentine microtubules, as well as the role of dentine "nano-tubules" that branch off from the primary microtubule will be discussed.

In an attempt to simulate *in-vivo* dentine exposure, samples of coronal dentine were treated with either deionized water (control) or a 1% w/w SnF_2 solution (pH 5.5) in a 5-day cycling model incorporating a daily 2 minute exposure to the SnF_2 treatment. The dentine was stored in artificial saliva for the remainder of the study. At the end of each day a specimen was removed and allowed to dry in air resulting in a control and 5 samples with exposure from 1–5 days. A dual-beam focused ion beam (FIB) methodology was developed to prepare consistently thin, cross-sectional specimens of dentin tubules for subsequent analysis. S/TEM-HAADF imaging and EELS/XEDS spectral imaging (SI) were used to determine directly the reacted product observed on the tubule surface following exposure to SnF_2. Figure 1(a,b) illustrates the FIB trenching and thinning to produce a thin, cross-sectional dentin lamellae. FIB sample preparation techniques will be discussed.

Following FIB sample preparation, a monochromated Titan™ 60-300 STEM equipped with a Super-X XEDS collection system was used to investigate the dentine tubules as shown in Figure 2(a). Characterization of multiple tubules revealed that they were unevenly covered by a coating on the inner tubule surface. Super-X XEDS spectral imaging of the various daily exposures revealed the coating was Sn-rich. The 1% w/w SnF_2 solution treated samples, shown in Figure 2(b), produced a Sn signal on all inner tubule surfaces, including that of the revealed "nano-tubule". The Super-X XEDS signal was then used to locate Sn rich regions for subsequent dual-EELS analysis to determine Sn oxidation state when compared against SnO, SnO_2 and SnF_2 powder standards.

The combination of FIB, Super-X XEDS SI, and dual-EELS has been applied successfully to characterize the reacted product formed from exposure of stannous fluoride to dentin with nanometer scale spatial resolution. Super-X XEDS spectral imaging showed clearly the location and presence of Sn and was used to identify regions for subsequent dual-EELS characterization. Dual-EELS was used to identify the oxidation state of Sn by comparing to Sn standards. This work has resulted in unparalleled characterization of dentine tubules and demonstrated that FIB, Super-X XEDS and dual-EELS are potent tools for characterizing salient nano-scale features in human dentine.

References:

[1] C.R. Parkinson, J.S. Earl, J. Clin. Dent. 20(**5)** (2009), p. 152-7.

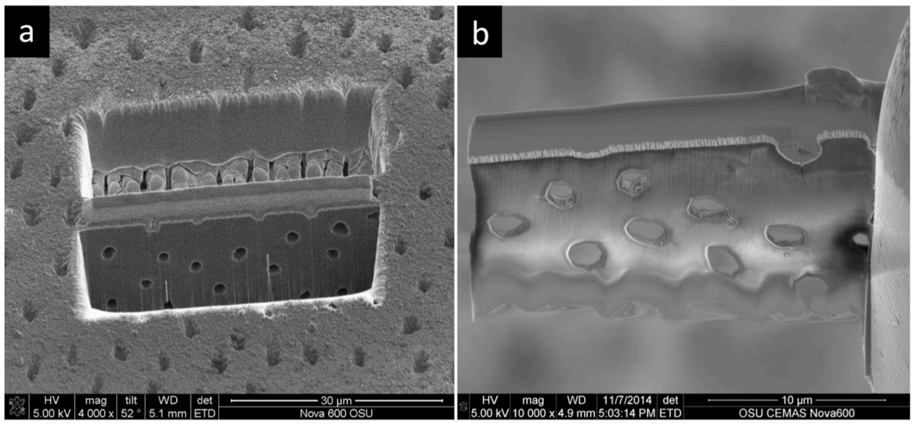

Figure 1. Secondary electron micrographs of 1% w/w SnF2 solution treated human dentine samples. (a) FIB preparation site with specific orientation (b) Thinned TEM sample

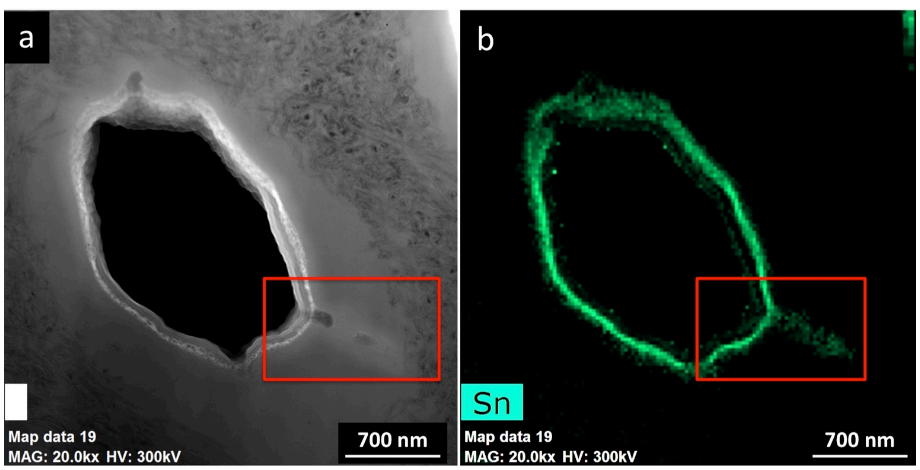

Figure 2. Super EDS on 1% w/w SnF2 Solution treated human dentine after 4 days. (a) HAADF STEM image of dentine tubule, (b) EDS Sn Map. The same region is pictured in (a) and (b), and the red rectangle surrounding a “nano-tubule” with the presence of Sn.