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Ablation and Microstructure Imaging of Dentin-Enamel Junction Using Focused Electron Beam in an Environmental Scanning Electron Microscope

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Focused electron beam or electron probe in an environmental scanning electron microscope (ESEM) is used to ablate and image a thin layer of mixed organic and inorganic interface between the human tooth's dentin and enamel phases. This thin layer is called the dentin-enamel junction (DEJ). Characterization of the DEJ properties, such as chemistry, width, morphology and microstructures, are critical for understanding tooth's biomechanical behavior, radiotherapy effect on the tooth, and other interfaces joining dissimilar materials [1, 2].

Chemically, the main component of human teeth is mineral hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$, which contains 39.89 wt% Ca. Enamel, the hard outer covering of the tooth, consists of more than 96 wt% inorganic hydroxylapatite, and the underlying dentin contains approximately 70 wt% hydroxylapatite. Compared to brittle enamel, the supporting dentin is more resilient, highly collagenous mineralized, and makes up the majority of the bulk tooth structure. Dentin has more biological components such as collagens and can absorb and distribute stresses from the enamel. The DEJ is a complex and critical structure bridging the enamel and dentin and preventing the propagation of cracks from enamel into dentin [3]. Previous studies indicate that there is a continuous gradient of organic components [1] and a transition zone of Ca distribution across the DEJ [4]. McGuire *et al*. [5] showed that type IV collagen exists at the DEJ. The estimated width of the DEJ from Raman microspectroscopy, energy dispersive spectrometry (EDS) and crack trajectory deviation varies significantly from a few μm [1, 6, and 4] to up to 150 μm [7]. Such large variations are likely related to the so-called functional width of the DEJ, i.e., the width of the DEJ differs depending on the property that is studied [2].

Morphologically the DEJ has a three-tiered structure, large scallops from 25 to 100 μm; microscallops from 2 to 5 μm; and a small scale microstructure [1]. However, morphology and small scale internal microstructures of the DEJ remain largely unrevealed. In order to expose the small scale internal microstructure of the DEJ and image the extracellular matrix under the scalloped surface of the DEJ, we employed FEI/Philips XL30 FEG-ESEM and strong electron beam energy to ablate the DEJ. The experimental conditions were accelerating energy 25 kV, spot size 4, ESEM mode, H_2O vapor pressure 1.5 Torr, and WD ~15 mm. The sample holder was cooled at 1ºC with a Peltier thermoelectric stage connected to a mini-chiller at 15ºC. Since the specimen is relatively large, its temperature may not be exactly 1ºC. At chamber pressure 1.5 Torr and temperature 1ºC, the relative humidity was approximately 30%. The specimens imaged were third molar halves of human teeth which had undergone previous decalcification and/or protein extraction exposing the dentin-enamel junction. After the first few images, the specimens were ablated or bombarded at the same location by the electron beam for approximately 10 min at a higher magnification. Then a series of images were taken at different magnifications. Figure 1 shows that the interior surface within a scallop can be removed by the focused electron beam. The four images shown were taken with a time interval of approximately 10 min. At a magnification of 8000X, the internal structure beneath the scalloped surface of the DEJ was then revealed (Figure 2).

References
[1] S.J. Marshall *et al*., J. European Ceramics Society 23 (2003), p. 2897-2904.
[2] M.P. Walker and B. A. Fricke, Dentin-Enamel Junction of Human Teeth. In "Wiley Encyclopedia of Biomedical Engineering", ed. M. Akay, John Wiley & Sons, Hoboken (2006), p 1061-1064.
[3] A. Nanci, Ten Cate's Oral histology: development, structure, and function. 7th ed. (2008), Mosby Elsevier, St. Louis.
[4] V.M. Dusevich *et al*., Microsc. Microanal. 18 (Suppl 2) (2012), p. 274-275.
[5] J.D. McGuire *et al*., J Dent Res. 93(10) (2014), p. 1028-34.
[6] C. Xu *et al*., Calcif. Tissue Int. 84(3) (2009), p. 221-228.
[7] X.D. Dong and N.D. Ruse, J. Biomed. Mater. Res. 66A (2003), p. 103–109.
[8] The authors acknowledge funding from NIH/NIDCR Grant R01DE021462.

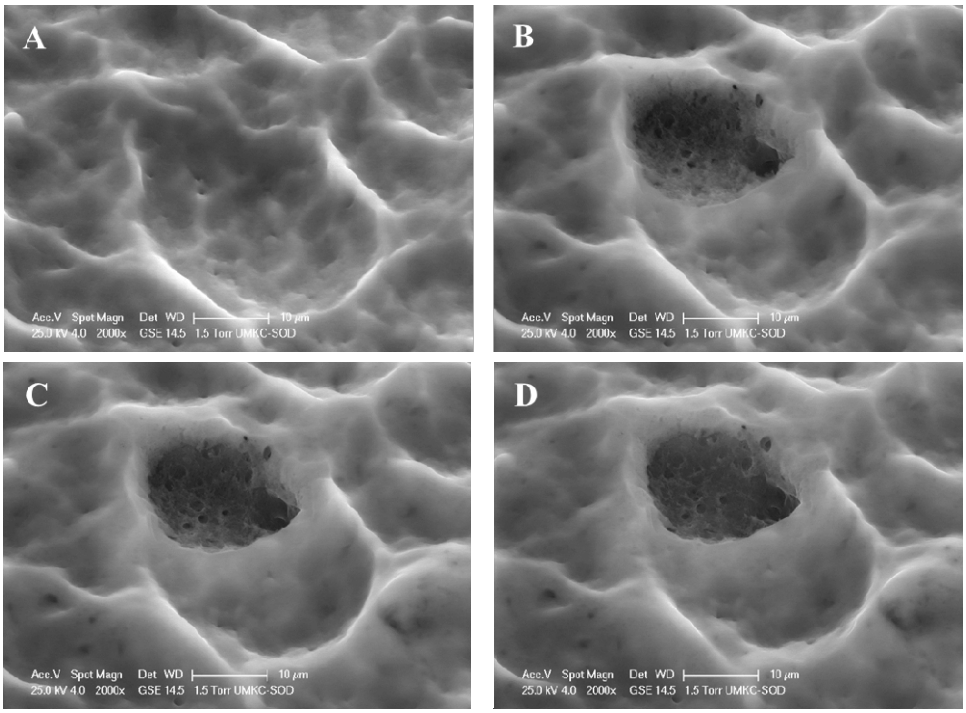

Figure 1. The interior surface of a scallop was removed and the internal structure beneath was revealed. Images A-D were taken at intervals of approximately 10 min between images.

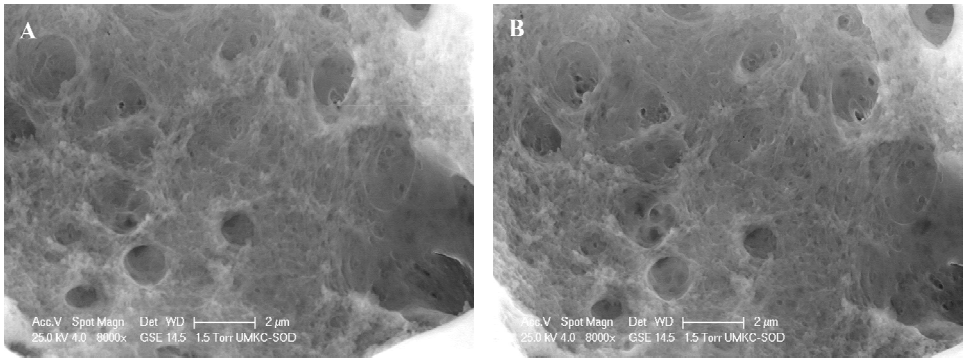

Figure 2. The underlying internal structure below the scalloped surface of the DEJ. Image A and B are zoomed in images corresponding to Image C and D in Figure 1, respectively. Differences in void are observed in the area off the center to the southwest direction.

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