

Structure-Property Characterization of the Dentine-Enamel Junction (DEJ)

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Abstract— The dentine-enamel junction (DEJ) is an important internal interface with strong and durable bonding between the hard outer layer (enamel) and the soft inner tooth core (dentine). This study investigated the microstructure of the DEJ by X-ray imaging method and synchrotron X-ray scattering techniques. Further high resolution elastic modulus mapping indicates that the DEJ is a band with a graded mechanical property rather than a discrete interface. The microstructure-property relationship is also illustrated. The knowledge of the architecture and properties of the natural DEJ will help in the biomimetic development of dental restorations and novel replacement materials and application techniques.

Index Terms—dentine-enamel junction, mechanical property, synchrotron X-ray scattering, microscopy

I. INTRODUCTION

FOR many decades now, the continued demand for improved dental treatment and prosthetics has driven the advancement of understanding of the micro-architecture of human teeth. The dentine-enamel junction (DEJ) is an important internal interface between the highly mineralized hard outer layer (enamel) and the softer tooth core (dentine). Unless diseased, this interface never fails by fracture or collapse, despite the extreme thermo-mechanical loading it

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experiences in the oral cavity. This stands in stark contrast to the interfaces between artificial dental restorative materials (fillings) and dentine [1]. The DEJ thus constitutes a superb lesson from nature on how to achieve strong, durable bonding between significantly dissimilar materials: the hard, brittle outer layer of enamel and the softer, but tougher dentine.

In this study a range of experimental techniques was employed for the purpose of structure-property analysis of this naturally engineered interface. The microstructure of the DEJ was firstly investigated by Micro-Computed Tomography (Micro-CT). Two modern high-resolution X-ray synchrotron scattering analysis techniques (SAXS/WAXS) were then applied in the undisturbed state to visualize the spatial distribution of HAp crystallites across the DEJ. The insight obtained into the thermal response of DEJ is briefly discussed in the end. The systematic experimental work reported here can be used to improve the understanding of the DEJ function in terms of its complex microstructure. The knowledge of the architecture and properties of the natural DEJ will benefit the biomimetic engineering of superior dental restorations and prosthetics, and the development of novel materials to emulate the DEJ.

II. STRUCTURE CHARACTERIZATION

A. Micro-CT reconstruction

Please check with your editor on whether to submit your The microstructure of the DEJ was firstly investigated by Micro-Computed Tomography (Micro-CT) and Environmental Scanning Electron Microscopy (ESEM) (see Fig. 1 a). The micro-CT system (SkyScan 1172 scanner, Kontich, Belgium) was used to obtain 3-D information about the tooth sample (including dentine, enamel and the DEJ). The high resolution scan was carried out at 0.6 μm resolution using 40kV voltage, 120 μA current and a 0.5mm aluminium filter. The resulting 3-D slices were reconstructed with SkyScan NRECON package and were shown in Fig. 1 b. the DEJ appears to form a complex shaped interface at the micrometer scale as observed in the 3-D reconstruction and also the 2-D topography contrast image (Fig.1 c). The contrast between dentine and enamel from Fig. 1 c indicates that the interface is not sharp [2]. This visualization further approves the micro- structural feature of the DEJ that is with a series of 25–100 μm diameter scallops [3] (see Fig. 1 d). The observation from micro-CT is important in establishing

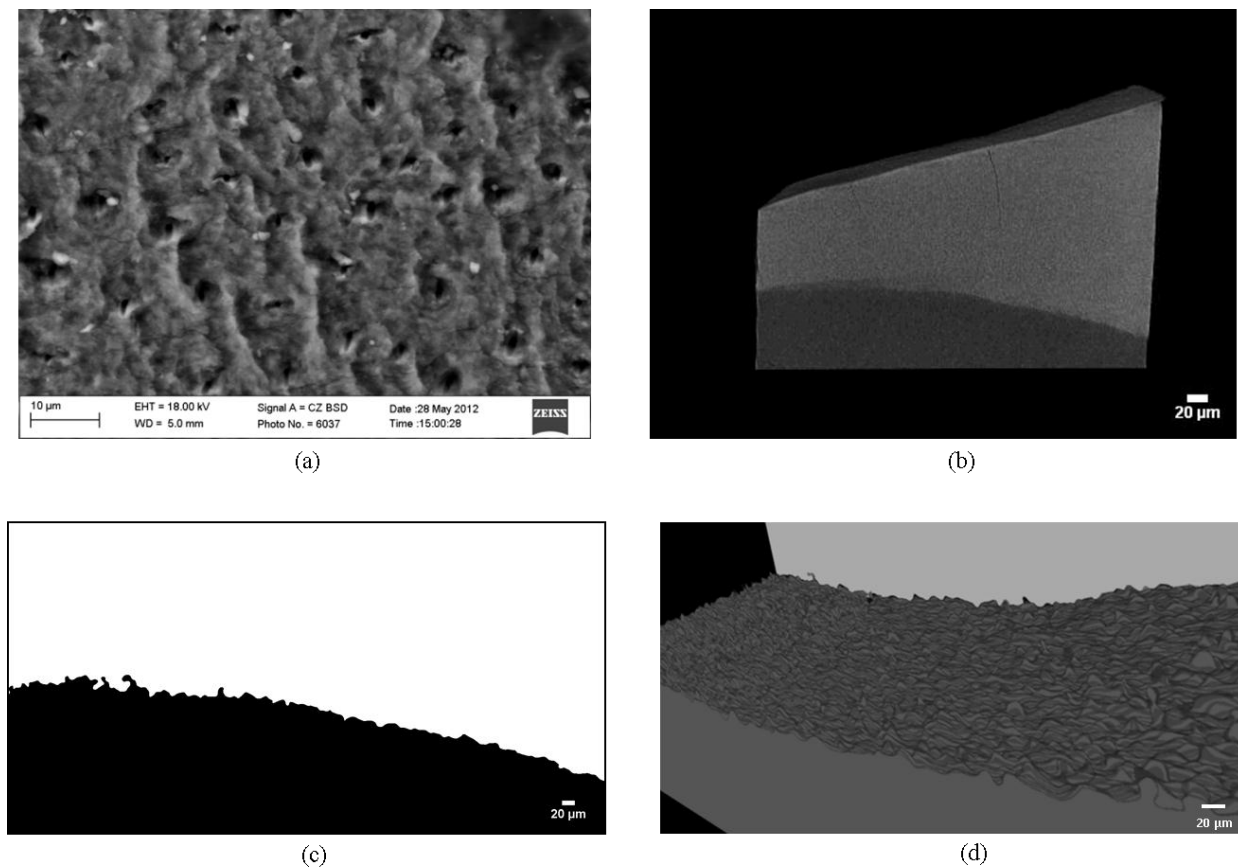


Fig. 1 Microstructural characterization of the dentine-enamel-junction (DEJ) (a) ESEM observation of scalloped structure; (b) 3-D reconstruction of DEJ sample; (c) 2-D projection of binary image of the DEJ; (d) scallop-like cross-section of the DEJ.

the basic structural understanding of the scallop-like structure of the DEJ, which may result in its remarkable mechanical behaviour. Scalloping is assumed to improve the bonding strength between enamel and dentine by increasing the interfacial area and it may lead to less stress concentration [4]. This indicates the requirement of examining the mechanical property across the DEJ, which will be discussed later.

B. Synchrotron X-ray scattering method I: Micro-beam WAXS mapping

The micro-beam X-ray diffraction experiments were performed on B16 beamline at Diamond Light Source (DLS, Oxford, UK) using monochromatic X-rays at 18 KeV. X-ray Eye detector (Photonic Science X-ray MiniFDI) was initially used by opening the slits to take the pictures of samples by radiography technique. It was used on the selection of the region of interest of samples with sharp interface across the DEJ in Fig.2 a in order to largely guarantee that the DEJ

plane of the selected area was almost parallel to the X-ray beam. Then the slits were closed and WAXS detector was exposed to collect WAXS pattern. The KB mirrors were used to focus the incident X-ray beam down to $2.7 \times 3.8 \mu\text{m}^2$, and thus, high resolution X-ray diffraction across the DEJ could be achieved and the results is shown in Fig.2 b.

The quantitative analysis of 2θ (scattering vector) of (002) peaks variation across the DEJ is able to reveal the

lattice parameter (c-axis) changes. The steep gradient over the DEJ thickness was collected based on the lattice parameters variation and was clearly visible in a range of 20 μm , which agrees with the observation in the Micro-CT scanning in Fig.1 c.

Furthermore, the bump was observed as shown in Fig. 2 b. The amount and depth of the scallops were found to vary between species, types of teeth and even between locations within a single tooth. In this case, the combination provides a consistent description of the DEJ spanning the range of length scales from the micro-scale down to sub-nano scale.

2-D mapping of structural transition of the DEJ (shown in Fig. 2 c) was then detected by medium resolution X-ray diffraction mapping with beam size focused down to $0.3 \times 0.3 \text{ mm}^2$ from the region of interest in the dental slice (marked in a rectangular in Fig. 2 a). Fig. 2 d presents the phase mapping by interpreting (002) peaks in dentine and enamel separately. There is a debate of whether the lattice parameter change of HAp crystallites during the growth of human dental tissue is attributed to chemical or mechanical perspective (e.g. residual stress). Most of the effort has been put on the chemical change [5]. However, the residual stress may also be associated with the natural growth process of each tooth, but no such quantification is currently available. Further examination by FIB-assisted DIC methods is needed in the future work.

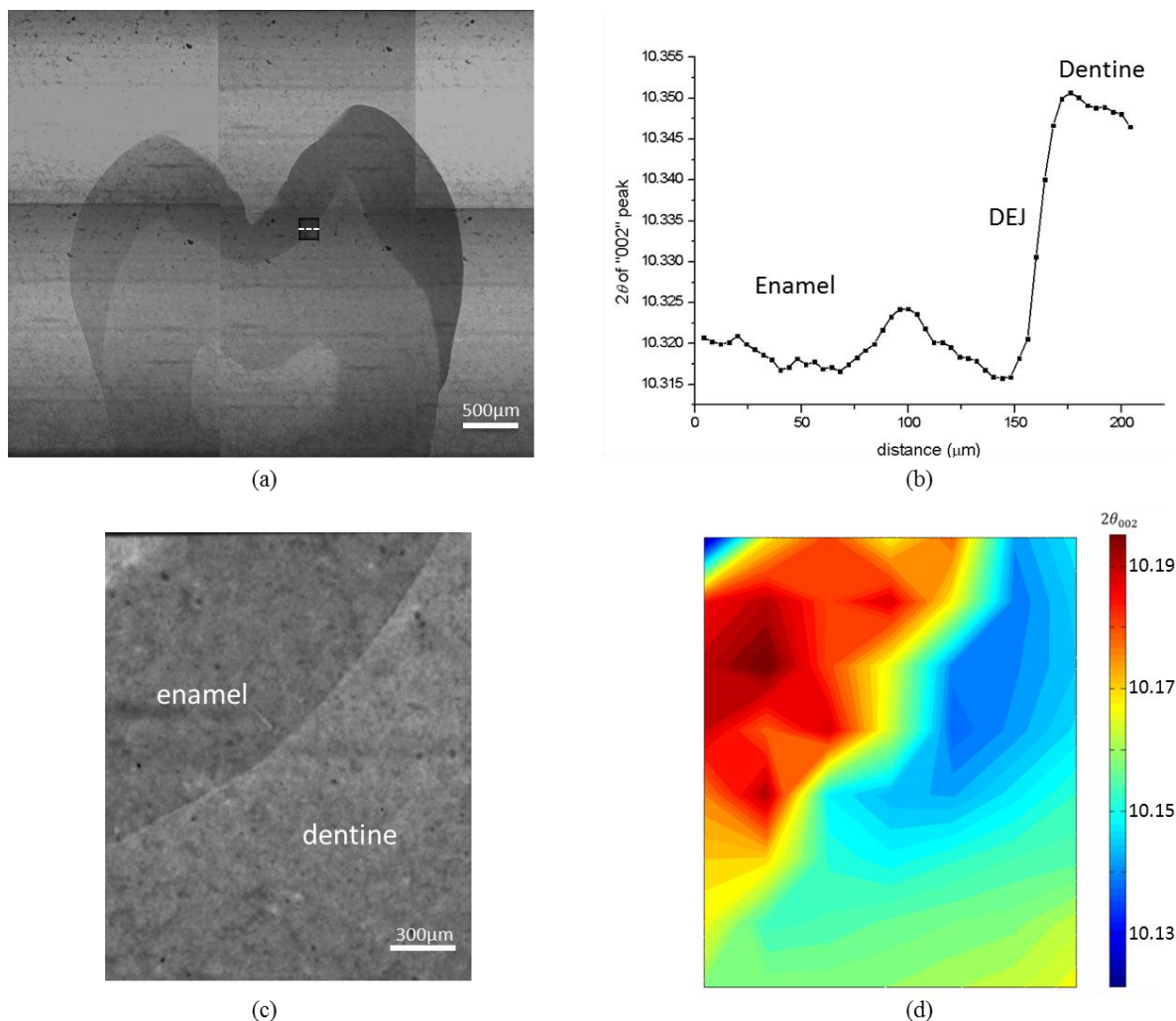


Fig. 2 Micro-beam X-ray diffraction mapping of dental slice; (a) radiographic image of dental slice; (b) high resolution line scan across the DEJ; the transition feature of the DEJ is clearly shown; (c) radiographic image of the region of interest (ROI); (d) phase mapping of dentine and enamel by interpreting (002) peak

C. Synchrotron X-ray scattering method II: Micro-focus SAXS mapping

Micro-focus SAXS experiments were carried out on I22 beamline at Diamond Light Source (DLS, Oxford, UK) using monochromatic 18KeV X-rays. The SAXS patterns were collected on the 2-D detector (Pilatus 2M, Dectris Ltd., Baden, Switzerland) positioned downstream of the sample with distance about 1040 mm. Compound lens were used to focus the incident X-ray beam down to the spot size of $14.5 \times 19 \mu\text{m}^2$. The dental slice illustrated in Fig. 3 a was repeatedly shifted in the sample plane perpendicular to the X-ray incident beam to collect a map of SAXS patterns.

The data analysis was applied following the procedure as described in section 3.1.4. The mean thickness and degree of alignment with orientation marked by black lines of HAp crystallites in the region of interest (see Fig. 3 a) including dentine, enamel and the DEJ are shown in Fig. 3 b-c as colour coded 2-D plot.

This provides an opportunity to map the structural variation in the plane of the dental slice. The gradient spatial distribution of the mean thickness is shown in Fig. 3 b.

Decreased value of the mean thickness was observed from enamel to the DEJ and further through the deep dentine. The 2-D spatial distribution of the degree of alignment and preferred orientation of the HAp crystalline nano-particles were visualized by SAXS as shown in Fig. 3 c. The enamel and dentine are readily distinguishable by their clearly different degrees of alignment. In detail, enamel has higher degree of alignment in the range of red colour (0.4-0.5), whereas dentine mostly locates at (0.1-0.3) from the colour bar. A region of increased alignment is found to lie just below the high gradient transition band close to the DEJ. The preferred orientation of HAp crystallites is shown by the black bars superimposed on the colour map.

In enamel, the particles are almost orthogonal to the DEJ plane. Same feature can be observed for the particles in dentine, but the particles gradually become parallel to the DEJ plane through the region near the DEJ. The variation of the structural properties of the DEJ suggests that a change in the internal nano- and micro-structure built into the structure optimizes the mechanical performance of dentine in the

vicinity of the interface with the much harder, highly mineralized enamel [7].

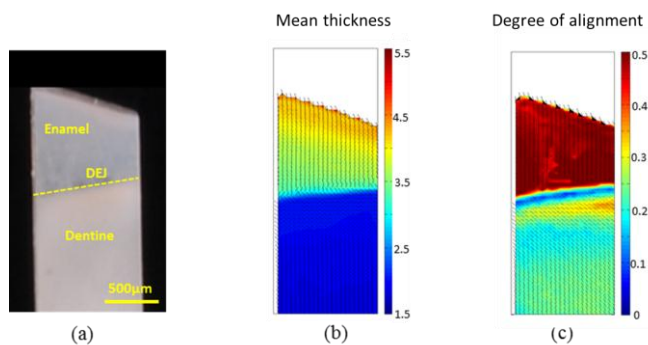


Fig.3 Micro-focus SAXS mapping of dental slice. (a) human dental slice of region of interest (ROI); (b) mean thickness and (c) degree of alignment mapping obtained by SAXS data analysis [6].

III. MECHANICAL BEHAVIOUR OF THE DEJ

A. Nanoindentation mapping of the DEJ

Nanoindentation mapping on the dental slice was carried out by a nanohardness tester (NHT) with a Berkovich diamond probe from CSM (Neuchatel, Switzerland). A load function composed of 30s loading, followed by 30s holding and 30s unloading was used under the force control feedback mode to a peak force of 2 mN. Mapping was performed on the region of interest (see Fig. 4 a) with scanning space about 60 µm apart both in the x- and y-axis.

Fig. 4 b and c demonstrate the results of the spatial distribution of reduced modulus and hardness respectively in the colour-coded 2-D maps of the region of interest. In general, contrast values were reflected in the colour ranges between dentine and enamel. In dentine, most regions have the reduced modulus below 40 GPa and hardness below 1 GPa, whereas in enamel, most regions have the reduced modulus in a range of 80-120 GPa and hardness in a range of 3-5 GPa. The DEJ is in the transition region with values gradually increased from dentine to enamel. However, it is found that the averaged reduced modulus of dentine was 20 ± 6 GPa and that of enamel was 70 ± 17 GPa. In addition, the averaged hardness in dentine was 0.7 ± 0.1 GPa, and 3.0 ± 1.3 GPa in enamel [6]. The hardness and reduced modulus of the control sample were consistent with the earlier nanoindentation studies on dental tissues [8,9].

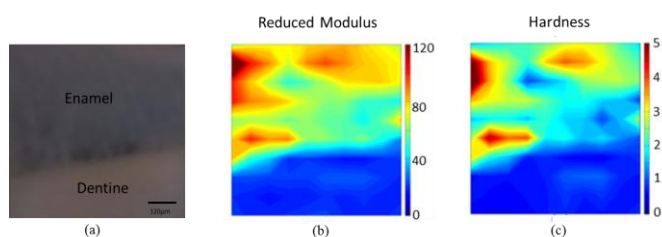


Fig. 4 Nanoindentation mapping of dental slice. (a) region of interest (ROI) in the human dental slice; (b) reduced modulus and (c) hardness obtained by nanoindentation data analysis [6].

B. High resolution elastic modulus mapping of the DEJ by CR-FM

The structure variation of the DEJ has been determined in last sections by X-ray imaging and scattering techniques. In order to study the local variation of the elastic properties in the region of the DEJ gradient (20 µm), contact resonance force microscopy (CR-FM) was used for the high resolution elasticity mapping of the scanned surface. Followed by the basic sample preparation procedure as described in [10], the DEJ samples were polished and stored under ambient conditions for further examination. Three scanning areas were selected as $30 \times 15 \mu\text{m}^2$, $10 \times 5 \mu\text{m}^2$ and $3 \times 1.5 \mu\text{m}^2$. To quantify the elastic modulus of the DEJ, a reference sample with the average value of elastic modulus of dentine and enamel (around 40 GPa) was selected (in this work, it is Mg) and needed to be examined as well.

Fig.5 coincides with the previous observation, where enamel (yellow coloured region) generally has higher elastic modulus than dentine (depicted in blue and green region), which is softer compared to enamel. This is also consistent with the results that obtained by nanoindentation (Fig. 4). Furthermore, it is found that, with the reduction of the scanning area, the mechanical property transition region becomes blurrier and smaller, despite the slight difference still visible between dentine and enamel. Such observation supports that the DEJ is a region with varied properties from enamel to dentine. In addition, most importantly, the DEJ should be seen as a graded band rather than a discrete interfacial line since gradual change in mechanical properties is the most important feature of the DEJ. Such graded transition from enamel to dentine may sustain higher loads than a direct bond between two distinct adhesive layers.

The observed values of the elastic moduli may have errors from the true values for the region of interest. However, the examination region is small and it is expected that the elastic modulus variation across the small region would not be significant. Therefore, the CR-FM mapping still provides valuable insights into the stiffness variation and arrangement of this remarkable interface.

IV. THERMAL BEHAVIOUR OF THE DEJ

Though considerable efforts have been directed at the study of structural and mechanical properties of the DEJ, improved understanding of the DEJ interface structure response to thermal treatment is still lacking. The effect of exposure of human skeletal tissues to elevated temperature is an important study topic in the context of forensic investigations, accident analysis, archaeology and paleontology [11]. However, an in-depth study of the heat-induced alterations of dental tissue remains lacking. Moreover, advanced high-energy techniques used in dentistry (e.g., dental lasers and light polymerizing units) may induce high temperature within teeth [12]. This provides additional need for detailed study of the effect of thermal exposure on the ultrastructural response of dental tissues. In particular, the DEJ is an important internal interface between the highly mineralized hard outer layer

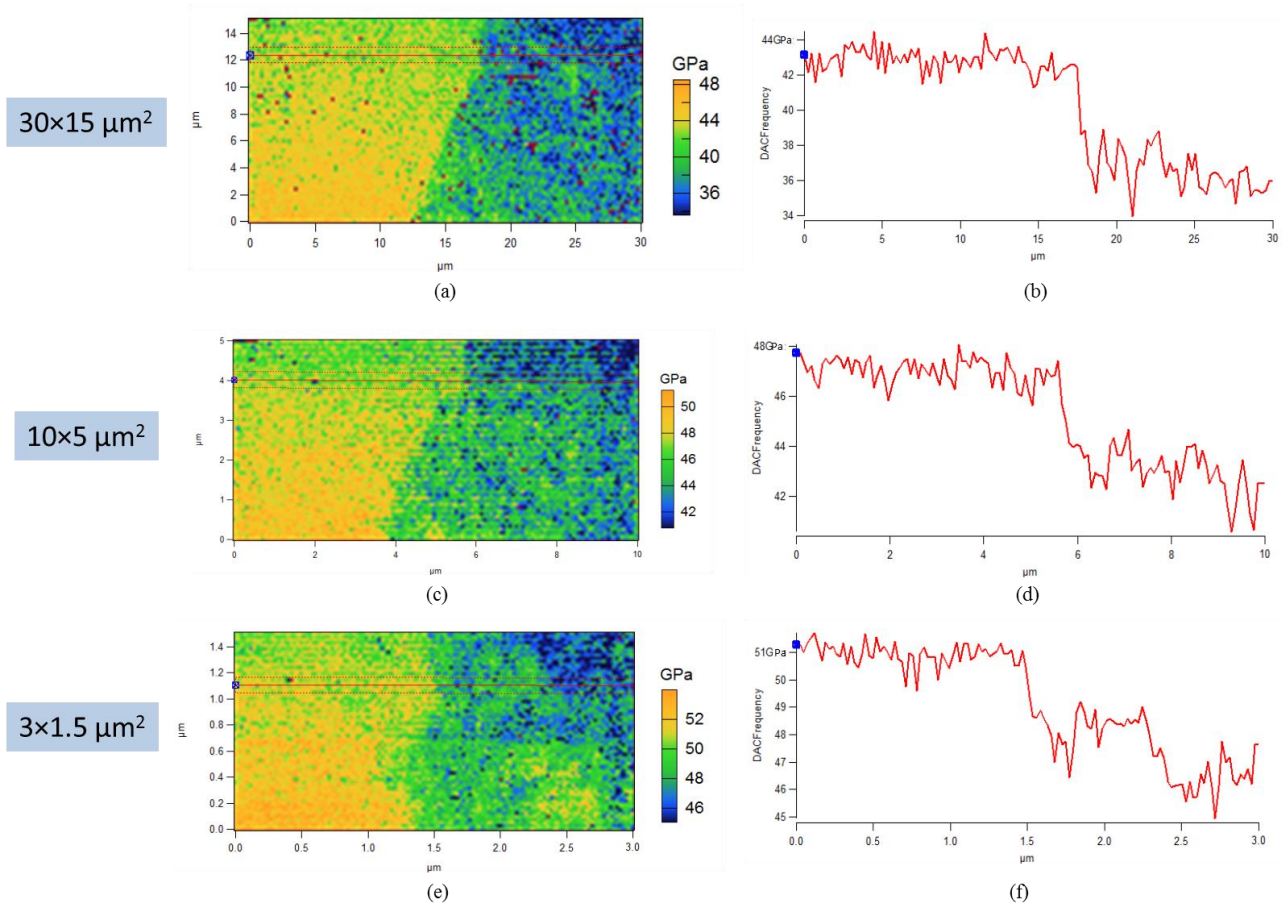


Fig. 5 Elasticity mapping of the DEJ in the area of $30 \times 15 \mu\text{m}^2$, $10 \times 5 \mu\text{m}^2$, and $3 \times 1.5 \mu\text{m}^2$. (a), (c) and (e) are higher resolution elastic modulus mapping of the DEJ by band excitation CR-FM; (b), (d) and (f) are line profile (red line in (a), (c) and (e)) of the elastic modulus of the DEJ.

(enamel) and the softer tooth core (dentine), showing a gradient [13]. Due to heat, a sudden detachment of the enamel can be seen around 500°C (see Fig. 6 a).

However, the detailed mechanisms are still unclear [14]. By examining the morphology of the DEJ exposed to different temperatures as shown in Fig. 6 b-f, it was found that the crack initiated at 400°C , and further propagated from 500°C to 700°C . The detachment occurred between dentine and enamel at 800°C .

The crack may be due to different thermal expansion coefficients, mechanical properties alteration during the thermal treatment or the structural changes around the DEJ (e.g. organic components in the dental tissue burnt off, resulting in no fibril connection any more between dentine and enamel). The different behaviours in terms of temperature observed in Fig. 6 for the entire dental slice and regular shaped sample are because the thermal distribution is more uniform in the regular sample when it is compared to the complex geometrical sample.

V. CONCLUSION

The microstructure of the DEJ was firstly investigated by X-ray imaging method (Micro-CT) and synchrotron X-ray scattering (SAXS/WAXS) techniques. The complex and scalloped structure at micro-scale was fully observed by 3-D

reconstruction techniques. Furthermore, the high resolution probing of the lattice parameter variation at sub-nano scale reveals the structural variation at the same range ($\sim 20 \mu\text{m}$) as has done by imaging. The 2-D spatial distribution of HAp crystalline nano-particles in terms of lattice parameters, mean thickness, degree of alignment and preferred orientation were also visualized.

In order to further examine the mechanical function region within the region of the DEJ, high resolution elastic modulus mapping was applied by band excitation CR-FM mapping. The results indicate that the DEJ is a band with a graded mechanical property rather than a discrete interface. The knowledge of the architecture and properties of the natural DEJ will help in biomimetic engineering of superior dental restorations and prosthetics, and the development of novel materials to emulate DEJ.

Interesting detachment of enamel phenomenon was captured in dental tissues during thermal treatment, and crack initiation and proration around DEJ further approved the significant structural alteration occurred during heating [6,15,16].

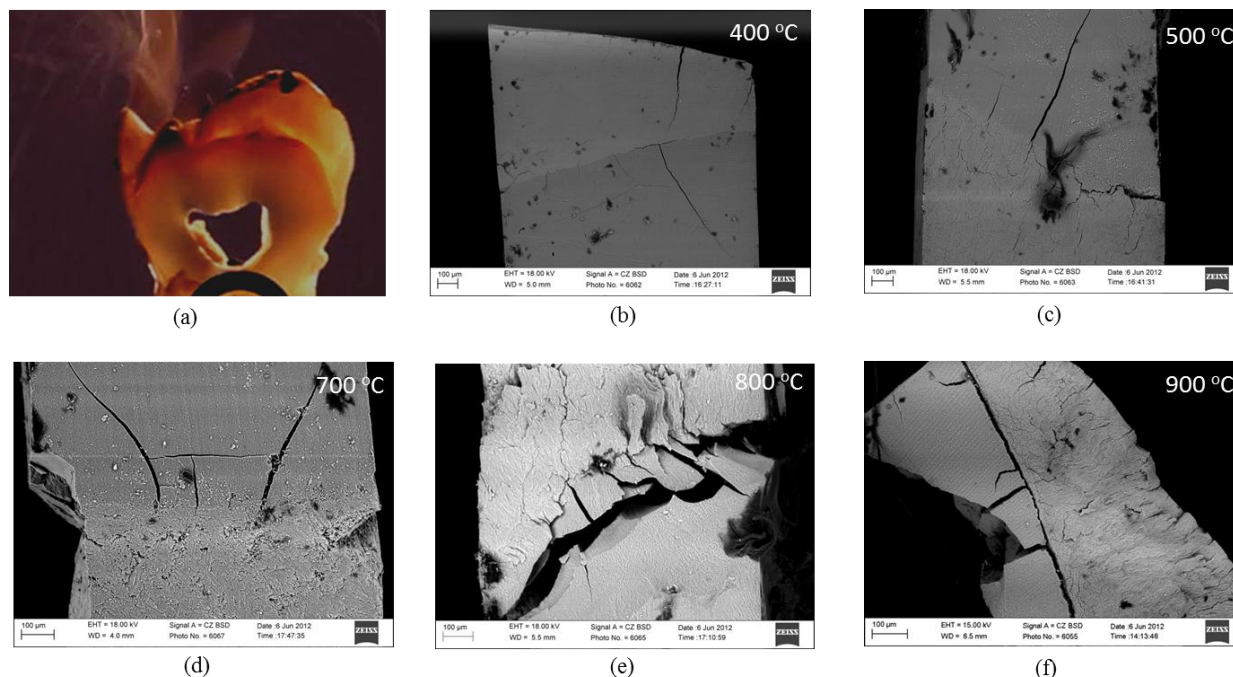


Fig. 6 (a) Sudden detachment in enamel near the DEJ at around 500°C; (b)-(f) Crack initiation and propagation around the DEJ observed at different temperatures.

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