Ultrastructural Changes in Burnt Dental Tissue Revealed by Synchrotron X-ray Scattering

Tan Sui, Michael A. Sandholzer, Nikolaos Baimpas, Gabriel Landini, A. Damien Walmsley, Philip J. Lumley and Alexander M. Korsunsky*

***Abstract*— The investigation of ultrastructural alterations of skeletal hard tissue exposed to thermal treatment has been proven to be crucial to obtain a reliable estimation of thermal exposure for forensic and archaeological study. However, there is only limited data on the heat-induced compositional and structural alterations of dental tissue. Visualizing and understanding the internal architecture of these materials is a challenging task that cannot be readily accomplished by conventional microscopy methods alone. We describe some experimental arrangements making use of synchrotron X-ray beams that shed light on the ultrastructure alterations of dental tissue. In particular, SAXS was used to investigate the alterations in thermally treated dental tissues, aiming at probing the ultrastructural changes of hydroxyapatite (HAp). The information about the internal architecture (the variation of crystalline size and the orientation of HAp nano-particles) of dental tissues was collected, providing a basis for estimating the maximum temperature exposure of forensic dentine samples.**

***Index Terms*— dental ultrastructure, small angle X-ray scattering, thermal treatment, forensic**

I. Introduction

The effect of exposure of human skeletal hard tissues to elevated temperature is an important topic of study in the context of forensic investigations and accident analysis. Much research has also been carried out into heat-induced changes that occur in bone, in connection with human remains studied in the context of archaeological and paleontological fieldwork [1-4]. However, the corresponding range and depth remains lacking in the study of exposure of dental tissue to fire. Only limited data is available for changes of entire teeth, and the ultrastructural differences in dentine and enamel have not been taken into account [5]. Furthermore, advanced high-energy techniques used in modern dentistry (e.g., dental lasers and light polymerizing units) may induce high temperature within the tooth [6]. This provides additional need for detailed study of the effect of thermal exposure on the ultrastructural response of dental tissue.

Multiple techniques have been used to study the ultrastructural heat-induced alterations of skeletal hard tissue. Most widely applied techniques are based on the absorption spectra of infrared radiation (Fourier transform infrared spectroscopy, FTIR), or X-ray diffraction (XRD) [1,5,7-9]. Although FTIR has been improved recently, there have been ongoing debates on the general limitations and validity of the crystallinity index (CI) quantified by FTIR [5,7,8]. Laboratory XRD usually involves the grinding of a sample and a subsequent volume-weighted averaged result, and does not provide local fine-scale changes of skeletal ultrastructure [10].

Synchrotron-based small angle X-ray scattering (SAXS) is widely used for the investigation of mineralized tissue ultrastructure, providing information at the nano-scale spatial resolution, thus helping understand the internal architecture of materials [11]. Compared with the conventional microscopy methods, SAXS allows a far higher throughput of samples with shorter time of analysis, the identification of local structural alterations at the nano-scale without the need to destroy the sample to prepare it for examination. The quantitative information obtained often has better statistical significance, allowing sensitive measurements of crystallite size and orientation to be obtained (compared to XRD) [12,13]. Recently, lab-based SAXS has been used to characterize the structural changes in human bone for forensic and archaeological purposes[10]. However, very few corresponding SAXS studies on dental tissues have been reported.

In this study, an improved analytical SAXS approach is used to provide new insights into the ultrastructural changes of dental tissues due to thermal treatment, and aims to develop a reference approach to the analysis of thermal history of heat-affected dental tissues.

II. MATERAILS AND METHODS

A. Sample Preparation

Four freshly extracted sound posterior human teeth (ethical approval obtained from the National Research Ethics Committee; NHS-REC reference 09.H0405.33/ Consortium

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R&D No. 1446) were cleaned to eliminate residues and rehydrated before the experiment. Fourteen tooth sections were prepared according to Märten et al. [14], with the individual section dimensions of $3\times1\times0.5mm^3$.

Tooth sections were subdivided into three different temperature groups with 30 min constant exposure at 400°C, 600°C, 800°C, whereas two unheated sections were used as controls. An ashing furnace (Carbolite AAF 11/3, Sheffield, United Kingdom) was used to generate the thermal stress. The teeth were removed as the desired duration of exposure was reached, and subsequently cooled down to room temperature. Progressive temperature-dependent shifts from a natural colour (control sample at room temperature) to dark brown (400°C), brown (600°C), and light greyish-blue (800°C) in dentine were observed, as shown in Fig.1.

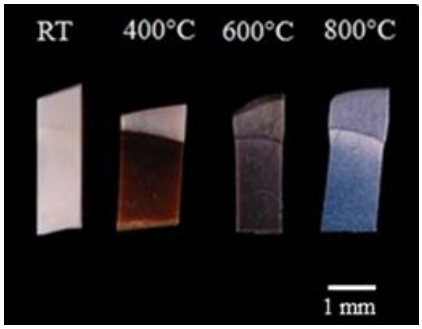

Fig. 1. Photography of dental slices after different thermal treatment.

B. Synchrotron X-ray Scattering Experiments

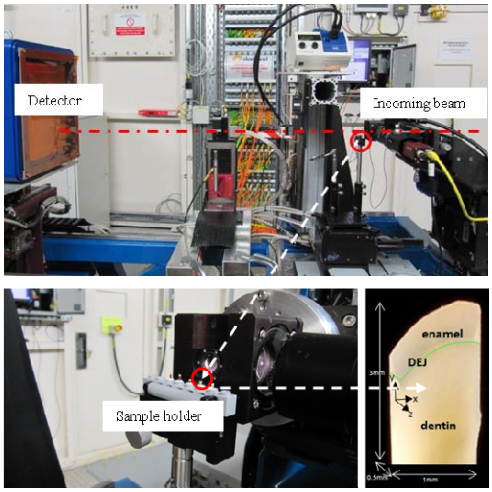

Fig. 2. Micro-focus SAXS set-up at I22 beamline in DLS

Micro-focus SAXS experiments were performed on the I22 beamline at Diamond Light Source (DLS, Oxford, UK) using monochromatic X-rays at the photon energy of 18keV. The distance between detector and sample was 1040mm, guaranteeing that clear and complete SAXS patterns could be captured on the 2D detector (Pilatus 2M, Dectris Ltd., Baden, Switzerland) positioned downstream of the sample. The use of the incident X-ray beam focused down to the spot size of $14.5\times19\mu m^2$ allowed to achieve the required spatial resolution. The sample was mounted upright in the air and ten scattering patterns were collected in transmission mode while the sample was repeatedly shifted in the lateral *x*-direction (longitudinal line scan) for each sample of control, 400°C, 600°C and 800°C. Moreover, transverse line scan (*y*-direction) was performed for 400°C, 600°C and 800°C samples, within a plane perpendicular to the X-ray incident beam travelling in the *z*-direction. The spacing between each two measurement points of line scan was 100 μm.

C. SAXS Data Evaluation

SAXS scattering occurs in the angular range of a few degrees from the incident beam direction. It is a coherent scattering phenomenon in which the scattering effect is mainly due to the variation of the electron density at the length scale of tens to thousands of angstroms (several nm to several hundred nm) within the material [15,16]. Quantitative interpretation of SAXS patterns provides insight into the mean thickness and degree of alignment of dense particles. For initial quantitative analysis, 2-D diffraction images are converted into 1-D intensity profiles and pre-processed using the Fit2D software package [17].

Mean Crystal Thickness

To determine the mean crystal thickness, the scattering intensity $I(q,\varphi)$ was radially integrated around the entire range of the azimuthal angle φ to obtain a function $I(q)$, where q denotes the scattering vector given in eq. (1) below [18,19]. For high values of q, $I(q)$ obeys Porod's law given by $I(q) = Pq^{-4}$, where P is the Porod constant. The general expression for P is given by [20]

$$P = \frac{S}{\pi V}\int_0^\infty q^2 I(q)dq \qquad (1)$$

where V is the total volume of the crystals, and S is their total surface area. Thus, without any assumption for the shape of the crystals, "mean crystal thickness" T given by $T = 4V / S$ [21,22] is evaluated as follows:

$$T = \frac{4}{\pi P}\int_0^\infty q^2 I(q)dq \qquad (2)$$

If a particular shape is assumed, namely, needle or platelet, T can be interpreted as an average measurement of the smallest dimension of crystallites [18].

Degree of alignment of Crystal

For the evaluation of the particle orientation in dentine, the degree of alignment ρ is used to describe the percentage of the aligned particles using the definition:

$$\rho = \frac{I_{oriented}(q,\varphi)}{I_{total}(q,\varphi)} = \frac{A_{oriented}}{A_{oriented} + A_{unoriented}} \qquad (3)$$

where $I_{total}(q,\varphi)$ is the total scattering intensity composed of the contribution from both the randomly oriented particles $I_{unoriented}(q,\varphi)$, and from aligned particles $I_{oriented}(q,\varphi)$ [23].

In order to quantify the degree of alignment, the SAXS patterns were integrated along all concerned scattering

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vectors q, which resulted in a function $I(\varphi)$ with the azimuthal angle φ [21,24]. The degree of alignment was calculated by the ratio of peak area to the overall area under the curve $I(\varphi)$, where A_{total} is the total area below the curve and $A_{oriented}$ is the area of the two peaks above the constant background level. Thus, the value of ρ ranges from 0 to 1, with $\rho = 0$ indicating no predominant orientation within the plane perpendicular to the incident beam, while $\rho = 1$ indicates a perfect alignment of all crystals [21,24].

III. Results

Mean Crystal Thickness

The mean thickness variation for longitudinal direction scan across thermally treated dental slices (400°C, 600°C, 800°C) as well as the control group is shown in Fig.1. Each point represents the average over the results from ten point measurements in dentine. The mean thickness of crystal in dentine increases from 2.01±0.09nm in the control sample to 2.81±0.10nm at 400°C, 4.90±0.16nm at 600°C and 4.88±0.16nm at 800°C.

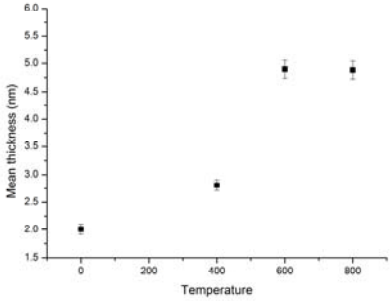

Fig. 3. Mean thickness variation of dentine with the increasing temperature of exposure.

Fig.4 and Fig. 5 display the spatial variation of thickness in the longitudinal and transverse direction scans in the 400°C, 600°C and 800°C samples. In Fig.4, the results from the control sample are also shown in order to highlight the relatively large variation between different temperatures. It is clear that the thickness variation with position is significantly smaller than its change with the temperature increase. This leads to the preliminary conclusion that some sintering of crystallites takes place with increasing temperature.

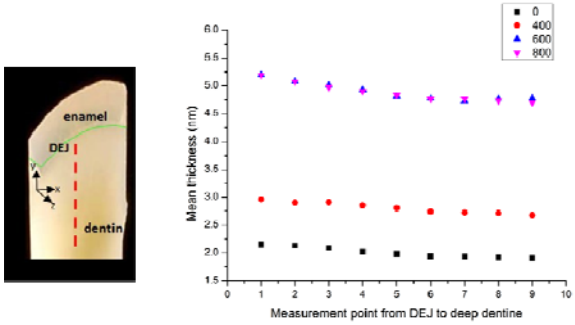

Fig. 4. Mean thickness variation in the longitudinal direction scan in dentine with increasing temperature. "0" denotes the control sample that remained at room temperature.

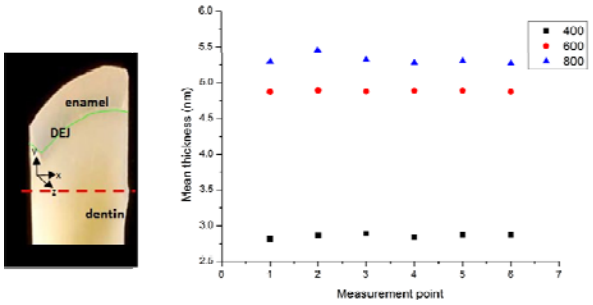

Fig. 5. Mean thickness variation in the transverse direction scan in dentine with increasing temperature.

Crystal Degree of Alignment

The variation of the degree of alignment of crystallites in the longitudinal direction scan in the thermally treated dental slices (400°C, 600°C, 800°C) as well as in the control group was determined, and is visualized in Fig.6 that represents the average results over ten scanning points in dentine. The degree of alignment of crystallites in dentine decreases from 0.27±0.02 in the control sample to 0.26±0.01 at 400°C, 0.21±0.03 at 600°C and 0.16±0.01 at 800°C. This indicates that the percentage of aligned particles gradually decreases as the temperature goes up. The relatively big error bar indicates that some variation is observed between the scanning points in the longitudinal direction scan.

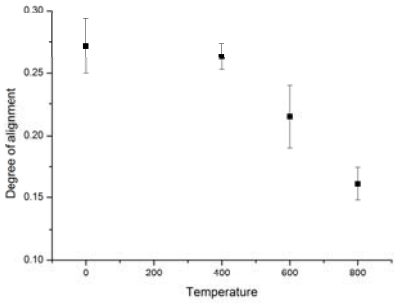

Fig. 6. Degree of alignment variation of dentine with increasing temperature

In detail, Fig.7 reveals how the degree of alignment varies longitudinally in the representative control sample and the 400°C, 600°C and 800°C samples. A significant change close to the dentine-enamel junction (DEJ) region is noticeable, both in the control sample and in the 400°C, 600°C and 800°C samples. In the distant dentine, the degree of alignment appears to be less variable.

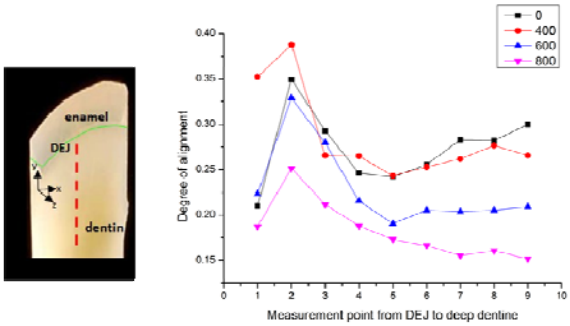

Fig. 7. The degree of alignment variation in dentine in the longitudinal direction scan with increasing temperature.

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In the transverse direction, the results from the samples treated at 400°C, 600°C and 800°C are selected. Slight variation indicates that the percentage of aligned particles remains almost constant, but with different levels depending on the thermal treatment.

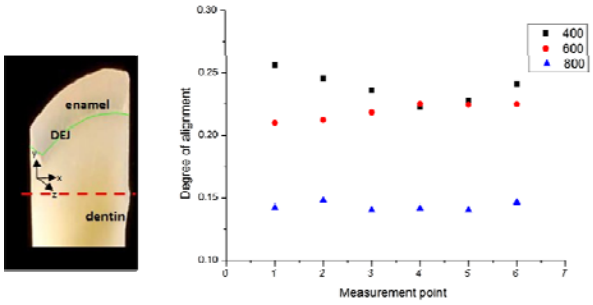

Fig. 8. The degree of alignment variation in the transverse direction scan in dentine with increasing temperature.

IV. DISCUSSION

Mean Crystal Thickness

The mean crystalline thickness (T) of the control samples (2.01 ± 0.09 nm) is consistent with the earlier transmission electron microscopy (TEM) studies and XRD data [5,25]. Compared with the original size at room temperature, the SAXS-derived increase of the mean crystalline thickness in the bones was found to be larger than that established for dentine in the present study [5,10,26]. Moreover, a non-uniform thermal treatment of the entire tooth may cause spatial variation that is expected to affect the results [5,10].

Further analysis of the variation in the longitudinal direction scans indicates that the slight reduction of the thickness may be due to the gradient in the properties of human dentine with the distance from the dentine-enamel junction [24]. Small variation in the transverse scan indicates that the change in this direction is significantly lower.

Crystal Degree of Alignment

The degree of alignment in the control sample of dentine (0.27) shows an almost random orientation of HAp crystallines, which is consistent with earlier SAXS results for dental tissue [14]. It is interesting to note that there is a decrease in the degree of alignment under different thermal treatments. This may be explained by the gradual disappearance (burn-off) of the organic phase. Since the organic phase serves as the support of the structure, this may result to the rearrangement of crystallites associated with rotation or anisotropic sintering.

It is also noted that the degree of alignment varies depending on the measurement location. Further examination along the transverse direction indicates a minor variation that is consistent with the material internal architecture, that is characterised by the random arrangement of HAp crystallites around tubules, prominent structural features of the dentine material [27]. The observed variation along the longitudinal direction, characterised by the reduction in the degree of alignment in the dentine around the DEJ, indicate the special structural variation in the dentine close to the interface with enamel. In contrast, in the distant dentine the degree of the degree of alignment appears less variable. The variation of structural properties of dentine close to the DEJ can be explained by the change in the internal nano- and micro-structure in order to optimise the mechanical performance of dentine in the vicinity of the interface with the much harder, more mineralised enamel material [14].

V. CONCLUSION

The effects of heat treatment on human dentine were successfully explored using small angle X-ray scattering (SAXS) technique. Quantitative analysis of SAXS patterns allowed establishing the variation of crystalline size and the orientation (degree of alignment) of HAp nano-particles in response to thermal treatment. The results indicate the temperature-dependent increase of the mean thickness of HAp crystallites, and the decrease of degree of alignment in dentine with the temperature of exposure. Significant variation of properties was also observed in longitudinal scans, i.e. in the direction perpenticular to the DEJ. Therefore, the ultrastructural changes observed in the present study can be used to obtain reliable estimations of the maximum temperature of thermal exposure.

In conclusion, synchrotron-based SAXS has been shown to be a powerful method for the determination the variation of the properties of HAp crystallites in human dentine induced by thermal treatment. Further study will be continued to investigate the dental ultrastructure by area mapping the entire slice. In addition, the results help characterise the changes of the ultrastructural features in response to thermal treatment, and offer interesting prospects for the use of this approach in the context of forensic analysis. Ultimately, the approach developed in the present project will also allow further design and optimization of treatment strategies for clinical applications, and provides an effective approach to deducing the heating history for samples of human dental tissue.

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