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Hierarchical Modelling and X-ray Analysis of Human Dentine and Enamel

Tan Sui, Michael A. Sandholzer, Nikolaos Baimpas, Alexander J.G. Lunt, Igor P. Dolbnya, Jianan Hu, Anthony D. Walmsley, Philip J. Lumley, Gabriel Landini, and Alexander M. Korsunsky*

Abstract—Human teeth consist of enamel, dentine and cementum, hierarchical mineralised tissues with a two-level composite structure. The understanding of the mechanical behaviour of dentine and enamel in terms of their micro- and nano-scale structure has been somewhat limited. Here we present an overview of our recent work aimed at improving the understanding of the internal lattice strain response of the mineral crystallites of different orientations under external in situ loading. A range of experimental techniques was employed for the purpose of this analysis. Small- and Wide- Angle X-ray Scattering (SAXS/WAXS) were used to determine the internal lattice strain and orientational distribution of HAp crystals, while quantitative stress distribution evaluation in the birefringent mounting epoxy surrounding the sample was carried out in parallel using photoelasticity. Finite element analysis and advanced multi-scale Eshelby inclusion modelling were used to interpret the data. The satisfactory agreement achieved between the model and the experimental data, in terms of the values of multi-directional strain components under the action of differently orientated loads, demonstrates that our multi-scale approach captures successfully the structure-property relationships between the hierarchical architecture of human dental tissues and their response to the

applied forces. Our systematic approach can be used to improve the insight into the mechanical properties of dentine and enamel, and of textured hierarchical biomaterials (such as bones) in general.

Index Terms— dental tissues, small- and wide angle X-ray scattering, multi-scale modelling

I. INTRODUCTION

HUMAN teeth are composed of dentine and enamel and cementum. These are hydrated biological mineral composite tissues with a hierarchical structure and versatile mechanical properties (Fig. 1). At the meso-scale the dentine microstructure has a notable feature of dentinal tubules, while enamel consists of a stacking of interlocked aligned prisms or rods [1,2]. At the nano-scale, both dentine and enamel contain organic matter that bonds together hydroxyapatite (HAp) crystals, but differ in terms of the overall stiffness, due to the variation in the volume fraction and the geometry of HAp crystals. Understanding the mechanical properties of complex, hierarchically structured tissues helps develop insight into how the internal architecture of these materials can determine the remarkable properties of dental materials.

Over half a century, research has been carried out on the mechanical properties of human dental tissues at the macro-scale [3,4]. However, until very recently few studies have focused on the influence of the nano-scale structure. This gap in the previous literature suggested a demand for further investigations using advanced experimental techniques and systematic modelling approaches to establish a deeper understanding of this multi-level structure based on the detailed knowledge of the internal architecture and hierarchical properties.

Synchrotron-based Small- and Wide- Angle X-ray Scattering (SAXS/WAXS) are advanced non-destructive techniques widely used to study the structure and elastic behaviour of composites. SAXS/WAXS have been applied quite recently to study the mineralized biological composites such as bones and bovine teeth [5-7] but these studies were carried on non-human samples. Moreover, previously reported studies did not take into account the nanoparticle distribution effect on the mechanical response. Profound understanding of the relationship between the nano-scale structure and the macroscopic mechanical behaviour remained lacking.

A number of composite models have been proposed to describe the mechanical interaction of different phases, e.g. the Voigt and Reuss bounds. However, these models mainly focused on the analysis of deformation in only one direction

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(the loading direction) and therefore were not able to provide adequate consideration of the elastic anisotropy. Recently, multi-scale Eshelby model has been used in dental tissue research [8-10]. It was shown to capture the micro-mechanical response reasonably well using the two-level hierarchical description.

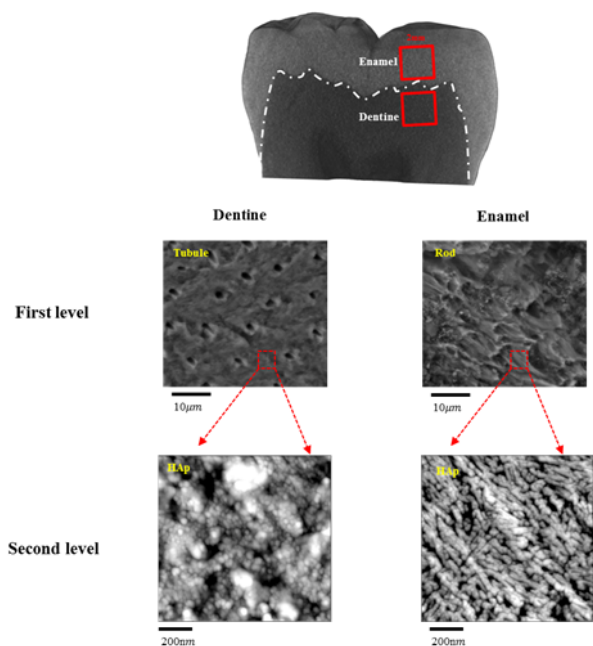


Fig. 1. Hierarchical structure of dental tissue

In the present review, *in situ* synchrotron SAXS/WAXS and photoelasticity techniques were applied simultaneously to measure the applied stress and the elastic lattice strain within the HAp crystallites of a human enamel and dentine samples. Multi-scale Eshelby models were established to describe the hierarchical structure of the human dental tissues and to predict their elastic behaviour.

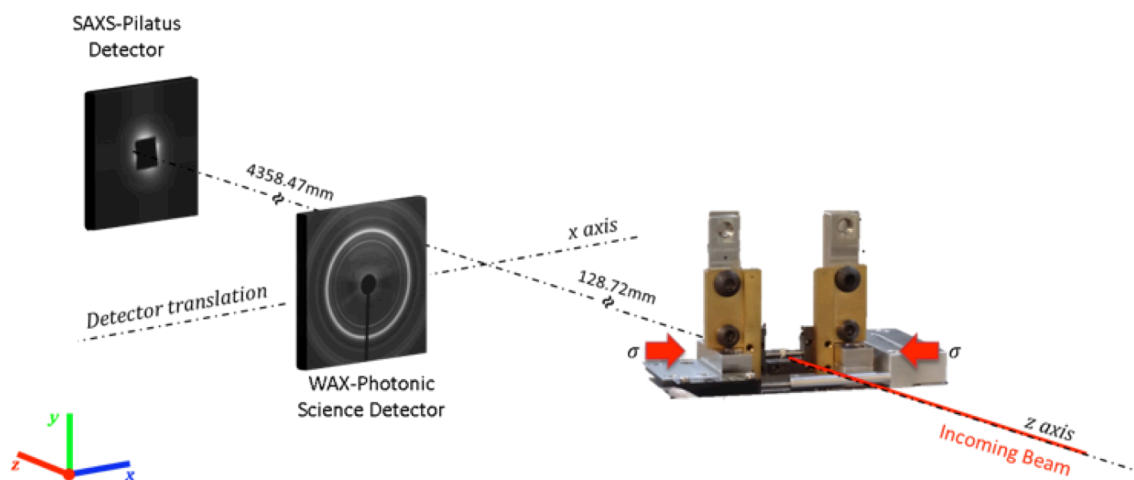


Fig. 2. Simultaneous WAXS/SAXS experimental set-up [8].

II. MATERIALS AND METHODS

A. Sample Preparation

Freshly extracted sound human third molars (ethical approval obtained from the National Research Ethics Committee; NHS-REC reference 09.H0405.33/Consortium R&D No. 1465) were washed and mechanically cleaned in distilled water to eliminate residues and kept in a -20°C freezer for a maximum 14 days before the experiment. The samples were rehydrated using distilled water and 2mm thick human dentine and enamel disks were cut just using a low speed diamond saw (Isomet Buehler Ltd., Lake Bluff, Illinois, USA). The disks were further cut into smaller bars and a series of polishing papers were used to produce the final 2×2×2mm cube of human dentine and enamel. For the purpose of planning the measuring positions and determining the precise loading cross-sectional area of dentine and enamel prismatic samples, a commercial micro-CT system (SkyScan 1172 scanner, SkyScan, Kontich, Belgium) was used, with 1.9µm isotropic resolution and 40kV voltage, 120µA current and a 0.5mm Aluminium filter.

For the photoelastic measurement, the samples were further embedded in the centre of a 12mm diameter, ~2.5mm thick disks of epoxy resin (Buehler Epokwick, ITW Test & Measurement GmbH, Dusseldorf, Germany). The disk side surfaces were subsequently polished to expose the samples. Finally, the cubic sample was kept for a maximum of 7 days in distilled water in a commercial fridge at 4°C until the experiment was performed.

B. In-situ Synchrotron X-ray Scattering Experiments

The experiments were performed on the B16 beamline at Diamond Light Source (DLS, Oxford, UK). Cube samples of human dentine and enamel were slowly deformed under compressive loading using a remotely operated and monitored compression rig (Deben, Suffolk, UK) with a 5KN calibrated load cell.

A monochromatic X-ray beam was collimated to the spot size of $0.5 \times 0.5 \text{ mm}^2$ and directed at the sample perpendicular to the loading direction. WAXS diffraction patterns were recorded using a Photonic Science Image Star 9000 detector (Photonic Science Ltd., UK) and SAXS patterns were collected by a Pilatus 300K detector (Dectris, Baden, Switzerland) placed further downstream of the X-ray beam. The experimental set-up is shown in Fig. 2. A disk of silicon powder and dry chicken collagen inserted beside the sample position were used as standards for calibration and to determine the sample to detector distances.

A Sharples S-12 demonstration polariscope was used to collect the photoelastic images, initially of an ‘empty’ epoxy disk as a calibration, and then of the epoxy disks with embedded enamel samples. The setup consisted of a light source, polarizers, quarter-wave plates and a digital SLR camera. A green-light filter was placed between the light source and the camera lens in order to obtain monochromatic images of the fringe patterns. Space restrictions meant that the sample had to be translated laterally from the WAXS configuration into the photoelastic set-up at each consecutive loading increment.

C. Data Evaluation

WAXS analysis relies on interpreting the shift of the diffraction peaks obtained from a bundle of HAp crystals, so that the average lattice strain in the crystals can be deduced [11]. Quantitative interpretation of SAXS patterns provides insight into the mean thickness and degree of alignment of dense particles [12]. The data analysis procedure involved pre-processing the 2D diffraction using Fit2D [13]. The detailed description of data analysis has been explained and illustrated in the previously published works [8-10]. Photoelasticity analysis allows the determination of the Tresca stress distribution by counting the fringe numbers.

D. Modelling

a) Finite element model (FEM)

The finite element package ABAQUS[®] v.6.9 was used to simulate the experimental photoelastic patterns by computing the Tresca stress distribution, and post-processing the results to obtain fringe patterns. Direct comparison of the photoelastic patterns obtained from FEM and from experiment provides a way of verifying the macroscopic properties of the epoxy and of the stress distribution within the epoxy disk around the enamel sample.

b) Multi-scale modeling

The Eshelby inclusion theory leads to the derivation of the constitutive law for a non-dilute population of inhomogeneities (HAp crystals) embedded in a finite matrix [14].

Both human dentine and enamel have a hierarchical two-level composite structure as illustrated in Fig. 1, where the first level is represented by the typical well-oriented microstructure with an arrangement of tubules in the dentine and keyhole-like rods in enamel. Despite the differences at the first structural level, dentine and enamel have a similar structure in the second level that consists of partially aligned HAp crystals embedded in the isotropic organic matrix.

Based on the structure of human dentine and enamel, a two-level Eshelby inclusion model can be established. The schematic flow diagram of the two-level Eshelby model is illustrated in Fig. 3.

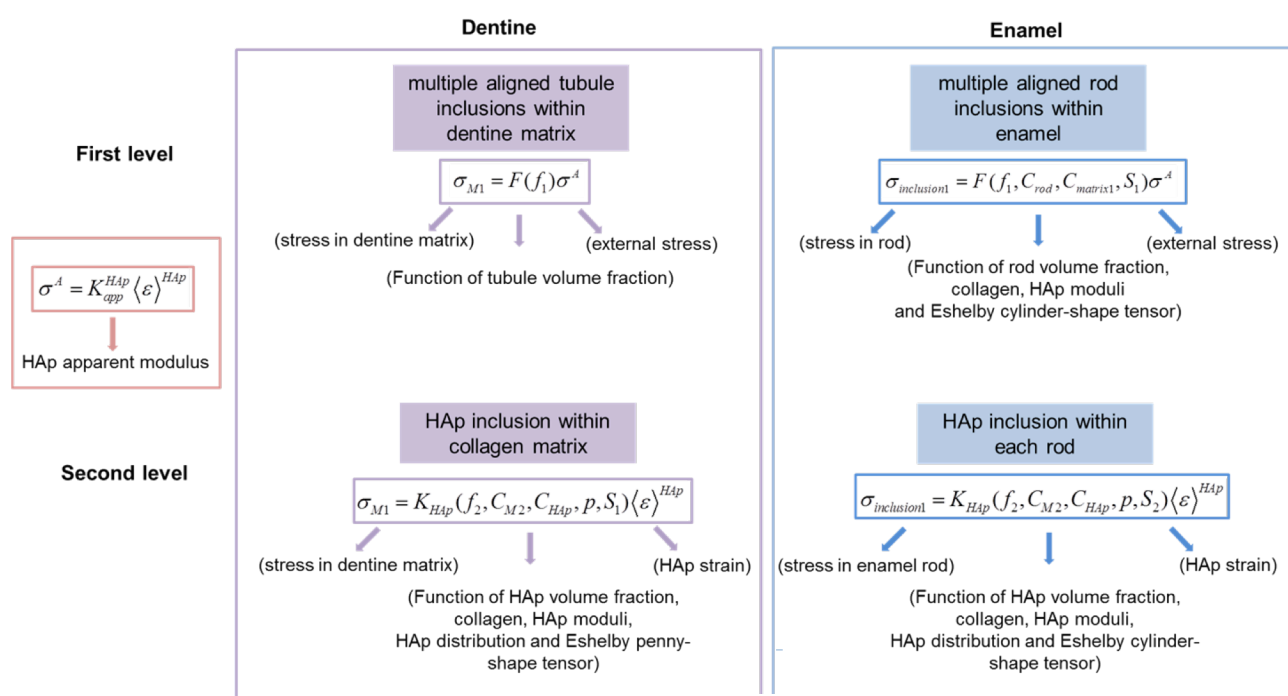


Fig. 3. Flow diagram of two-level Eshelby models for dentine and enamel.

In detail, the purpose of the first-level model is to establish the relationship between the externally applied stress σ^A and the stress in the organic matrix $\sigma^{matrix1}$ of dentine or the stress in the rod inclusions σ^{rod} of enamel. The second-level model is to establish the relationship between the stress of organic matrix in dentine or rod inclusion in enamel from the first level and the average lattice strain in the HAp crystals. Finally, by combination of two-level structure, the HAp crystals response to external macroscopic loading could be demonstrated by the improved Eshelby model, i.e. the apparent modulus (designated “K_model” in Fig.5 a-b)

III. RESULTS

A. Finite element simulation

The matched photoelastic fringe patterns for the ‘empty’ epoxy disk based on the computed Tresca stress distribution from ABAQUS simulation gives rise to the calibrated elastic constants of the epoxy disk. Following this, the fringe patterns and the Tresca stress distribution of the epoxy with embedded enamel samples at different loads were computed, and are shown in Fig. 4. The good matching ensured the extraction of the average stress state around the human dentine and enamel sample. This effectively uniaxial stress state served as the input for the two-level Eshelby model.

B. Eshelby model evaluation

In the model for dentine and enamel, the material properties and other parameters were taken from the literature and refined by fitting with the experimental data. A comparison of the applied compressive stress vs. elastic lattice strain for HAp of dentine and enamel is shown in Fig. 5 a-b. The experimental results indicate an approximately linear relationship in each direction as expected. It is found that the Eshelby model prediction lies between the two bounds (Voigt and Reuss bound) results and is closer to the experimental data.

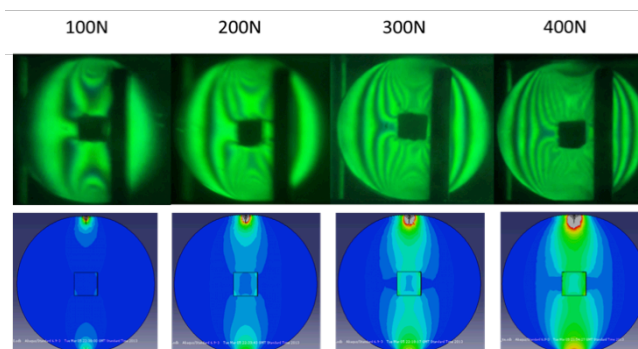


Fig. 4. Comparison of experimental photoelastic fringe patterns and the equivalent output from FEM simulation

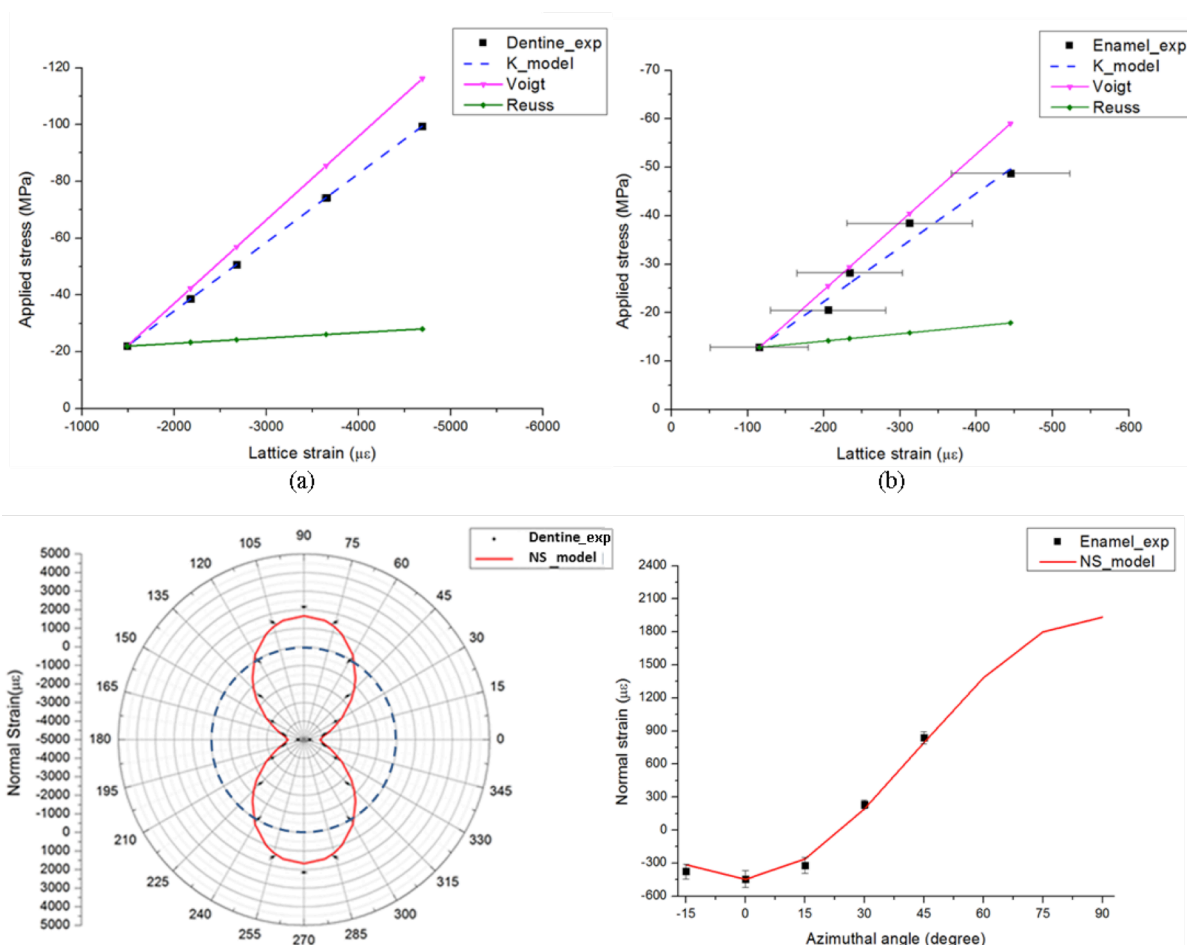


Fig. 5. Comparison of experimental data and modelling results: (a) Applied compressive stress vs. elastic lattice strain for HAp of (a) dentine and (b) enamel; Normal strain (NS) component variation with orientation distribution of (c) dentine and (d) enamel [8-10]

Furthermore, good agreement is observed in the variation of the normal strain components of the HAp crystals with respect to different azimuthal angles at the maximum external stress, predicted by the model, is shown in Fig. 5 c-d, together with the experimental data.

IV. DISCUSSION AND CONCLUSION

This study was conducted using the penetrating power of synchrotron X-rays to provide a bulk probe for structure and strain analysis. Unlike the vast majority of studies that rely on surface characterisation techniques (SEM, AFM, nanoindentation, Raman, etc.), this ensures that the effects of sample preparation (e.g. cutting and storage) are minimal, since they typically affect superficial layers of depths not exceeding $\sim 0.05\text{mm}$, i.e. a small proportion of the total sample thickness (2mm in our study).

The different mechanical responses of HAp crystals with respect to azimuthal angle shown in Fig. 5 originate from the strong anisotropic stiffness of HAp crystals. A validation can be demonstrated by examining the detailed effect of crystal orientation on the modulus. A three-dimensional representation of the directional modulus dependence for a single HAp crystal was calculated by transforming the transversely isotropic stiffness matrix of a single crystal using rotation matrices for different angles. The non-spherical shape illustrates the elastic anisotropy of the HAp crystal, as shown in Fig. 6. Here the x -axis represents the longitudinal direction of the HAp crystal.

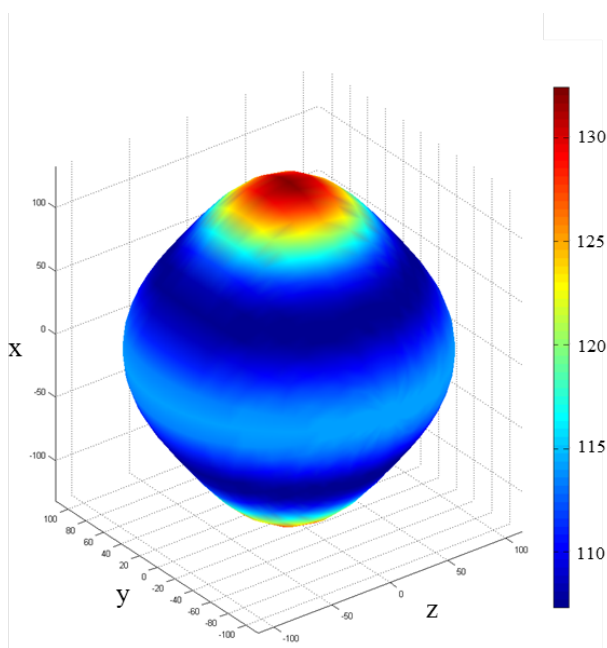


Fig. 6. Three dimensional representation of directional dependency of HAp elastic modulus (GPa)

In this review report, the lattice strain variations in the nano-scale HAp crystallites within human dentine and enamel were measured during *in situ* elastic compression by the combination of synchrotron SAXS/WAXS and photoelasticity techniques. In addition, multi-scale Eshelby inclusion models were established and validated as an numerical simulation technique that offers improvements

over the earlier proposed composite models [15,16]. It made it possible to estimate the elastic properties of human dentine and enamel on the basis of multi-scale consideration of their hierarchical structure. This systematic experimental and modelling approach offers a powerful tool for the evaluation of the multi-scale structural-mechanical property relations in human dental tissues, essential for understanding tissue degradation and the development of better prosthetic materials and dental fillings.

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