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Hierarchical modelling of in situ elastic deformation of human enamel based on photoelastic and diffraction analysis of stresses and strains

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ABSTRACT

Human enamel is a typical hierarchical mineralized tissue with a two-level composite structure. To date, few studies have focused on how the mechanical behaviour of this tissue is affected by both the rod orientation at the microscale and the preferred orientation of mineral crystallites at the nanoscale. In this study, wide-angle X-ray scattering was used to determine the internal lattice strain response of human enamel samples (with differing rod directions) as a function of in situ uniaxial compressive loading. Quantitative stress distribution evaluation in the birefringent mounting epoxy was performed in parallel using photoelastic techniques. The resulting experimental data was analysed using an advanced multiscale Eshelby inclusion model that takes into account the two-level hierarchical structure of human enamel, and reflects the differing rod directions and orientation distributions of hydroxyapatite crystals. The achieved satisfactory agreement between the model and the experimental data, in terms of the values of multidirectional strain components under the action of differently orientated loads, suggests that the multiscale approach captures reasonably successfully the structure–property relationship between the hierarchical architecture of human enamel and its response to the applied forces. This novel and systematic approach can be used to improve the interpretation of the mechanical properties of enamel, as well as of the textured hierarchical biomaterials in general.

1. Introduction

The increasing need to understand and predict the effect of structural alterations on the performance of dental tissues and their artificial replacements arises both in the context of clinical treatment, and the development of novel dental prosthetic materials [1]. The elucidation of the dependence of the mechanical behaviour of human enamel on its complex hierarchical structure remains a challenging task. Two different length scales dominate the structure and the mechanical behaviour of the enamel: At the microscale, ~5 μm diameter keyhole-like cross-section aligned prisms (or rods) are oriented towards the crown of the tooth [2]. At the nanoscale level, the rod is known to be a composite made from needle-like biological hydroxyapatite (HAp) crystals (~25–30 nm thick and of length known to be more than 1000 nm, or even spanning the entire thickness of the enamel layer) [3,4], which are held together by the protein matrix between the rods [5–7]. The orientation of HAp needle-like crystals within the rod is known to be a gradual variation on the length scale of the rod diameter [8,9].

The focus of most research to date has been on the mechanical properties of the enamel at the macroscale, with microstructural effects rarely taken into account [2,10]. Very few studies have focused on the multiscale analyses required to determine the influence of the nanoscale structure on the macroscopic mechanical response [11]. In fact, both the crystal shape and orientation of the mineral phase nanocrystals have previously been shown to have an effect on the anisotropy of overall stiffness and strength [12]. Therefore, in order to establish a firm understanding of this hierarchical structure–property relationship, further application of advanced nanoscale techniques and the formulation and refinement of systematic models are required.

Synchrotron-based wide-angle X-ray scattering (WAXS) is a non-destructive analytical technique used to quantify the internal strain of atomic lattices on (poly)crystals, both residually stressed and subjected to external loading in situ [13,14]. In addition, WAXS techniques are able to reveal quantitative information about the orientation distribution of crystals (texture) [15]. For example, recent applications of WAXS include the determination of the

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mechanical behaviour of mineralized biological composites such as bone and bovine teeth [14,16–18] while simultaneously providing insight into the crystallographic parameters and textures of such materials [8]. The strain distribution across the amelo-dentinal junction (ADJ) in bovine teeth was investigated by Almer and Stock [16]. However, this was limited to the strain in the loading direction [16,19]. In practice, the external mastication load is not perfectly aligned with the longitudinal direction due to the complex tooth geometries (e.g. on transverse ridges and cusps in first molars), and also due to the local orientation variation of HAp crystals within the rods. Therefore, the analysis of the relationship between the orientation of the applied load and strain needs to be sought. Moreover, the studies reported earlier did not take into account the nanoparticle distribution effect on the mechanical response. In addition, very few studies devoted to human enamel have ever been published [20]. It is therefore unsurprising that there is a lack of understanding of the effects of different growth histories, species or race on the mineralized tissue morphology, and the corresponding mechanical properties [21].

In order to carry out the in situ mechanical loading experiments in a versatile manner, i.e. allowing different directions of loading with respect to the preferred directions of structural orientation within the tissue, the sample was embedded in a photoelastic epoxy disk. The enamel cubes studied in our preliminary experiments could not withstand high external load applied by direct compression between the platens. Without the protection of the epoxy disk, samples were likely to develop local stress concentrations and/or microcracks that reduce the accuracy of measuring the mechanical behaviour of enamel. Furthermore, due to its birefringent properties, epoxy offers the possibility of deducing information about the stress distribution around the sample using photoelastic techniques. Photoelasticity is a non-destructive, whole-field method widely applied in stress distribution analysis. The fringe pattern arises when the sample is viewed between crossed polarizing plates, with the colour or brightness of the fringe being related to the difference between the principal stresses, which is in turn proportional to the maximum shear stress, or Tresca stress, in the material [22]. We combine the photoelastic analysis technique with WAXS analysis during in situ loading in order to establish the relationship between the external stress distribution and the internal crystal lattice strain.

A number of different models of composite deformation have been previously used to describe the elastic response of mineralized biological tissues that arises through the interaction between different constituent phases [11]. This approach also allowed the unknown properties of the component phases to be determined [23]. However, these models mainly focused on the analysis of deformation in only one direction (loading direction) and therefore were not able to provide adequate consideration of the elastic anisotropy. Recently, a multiscale Eshelby inclusion model has been established and successfully applied for the evaluation of the mechanical response of human dentine [12] and of the enamel subjected to compression along the longitudinal direction of the rods [19] by capturing the relationship between the nanoscale structure and the macroscopic loading. The multiscale modelling approach was shown to capture the micromechanical response reasonably well using the two-level hierarchical description of the structure of dentine and enamel, with each level consisting of an isotropic matrix and a group of anisotropic inclusions.

In this study, in situ photoelasticity and synchrotron WAXS techniques were applied simultaneously to measure the applied stress, the internal HAp crystalline orientation distribution and the elastic lattice strain for three samples of human enamel. The samples were prepared so that the primary rod direction was at a different orientation with respect to the external compressive loading that was applied diametrically to the epoxy disk containing the tissue sample (see Fig. 1). The multiscale Eshelby inclusion model was then applied to the analysis of the results, and the capability of the model to capture the relationship between the nanoscale structure and macroscopic loading was investigated.

2. Materials and methods

2.1. Sample preparation

Freshly extracted human third molars with no apparent damage, caries or other dental treatments were used for this study (ethical approval obtained from the National Research Ethics Committee; NHS-REC reference 09.H0405.33/Consortium R&D No. 1465). An enamel disk 2 mm thick was cut from the each tooth using a low-speed diamond saw (Isomet Buehler Ltd., Lake Bluff, IL, USA) and further prepared into smaller bars. A series of polishing papers were used to refine the final 2 mm × 2 mm × 2 mm cube of enamel. The cubes were placed in the centre of a 12 mm diameter cylindrical mould and embedded in epoxy resin (Buehler Epokwick, ITW Test & Measurement GmbH, Dusseldorf, Germany). The disks' surfaces were subsequently polished to expose the enamel surfaces.

In total, the three cubic enamel samples were prepared (designated #6, #7 and #3) with different rod directions with respect to the loading direction (x-direction in Fig. 1a). The predominant direction of rods in sample #6 was parallel to the loading direction with rods lying in the x–y plane, in sample #7 it was perpendicular to the loading direction with rods lying in the x–y plane, and in sample #3 it was perpendicular to the loading direction with rods lying in the y–z plane.

2.2. In situ X-ray diffraction measurements

2.2.1. Photoelasticity setup

A Sharples S-12 demonstration polariscope was used to collect the in situ photoelastic images. The setup consisted of light source, polarizers, quarter-wave plates and a digital SLR camera as shown in Fig. 1. The quarter-wave plates remained crossed and polarizers were aligned crossed to establish the dark field. A green-light filter was also placed between the light source and the camera lens in order to obtain monochromatic fringes to simplify the analysis. A solid epoxy disk (without the sample in the centre) produced from the same batch of epoxy resin was used as a common calibration specimen.

2.2.2. Mechanical loading setup

A schematic diagram of the experimental setup is shown in Fig. 1a. The epoxy disk which contained the cubic sample of human enamel was slowly deformed along the x-direction in laboratory coordinates (Fig. 1a). Compressive loading was applied along the x-direction at the load levels from 0 to 400 N using a remotely operated and monitored compression rig (Deben, Suffolk, UK) with a 5 kN calibrated load cell. The rig was equipped with custom-made jaws, allowing a high-energy transmission X-ray setup to be used. The load was incrementally increased (in 25 N steps and a loading rate of 3.8 N s^{-1}) and held constant while the WAXS and photoelastic patterns were collected.

2.2.3. Beamline diffraction setup

The experiment was performed on the B16 test beamline at Diamond Light Source (DLS, Oxford, UK). A monochromatic X-ray beam of 20 keV photon energy (wavelength: $\lambda = 0.062$ nm) was collimated by slits to a spot size of 0.5 mm × 0.5 mm. Radiographic images of the samples were initially used to align the samples and determine the position of interest. The incident beam on the