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Disruption of enamel crystal formation quantified by synchrotron microdiffraction

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ARTICLE INFO

Article history:
Received 25 May 2012
Received in revised form
29 August 2012
Accepted 30 August 2012

Keywords:
Dental enamel
Biomineralization
Texture
Synchrotron microdiffraction
Crystallite orientation
Mucopolysaccharidosis

ABSTRACT

Objectives: To understand the pathology of the ultrastructure of enamel affected by systemic disorders which disrupt enamel tissue formation in order to give insight into the precise mechanisms of matrix-mediated biomineralization in dental enamel in health and disease.
Methods: Two-dimensional synchrotron X-ray diffraction has been utilized as a sophisticated and useful technique to spatially quantify preferred orientation in mineralized healthy deciduous dental enamel, and the disrupted crystallite organization in enamel affected by a systemic disease affecting bone and dental mineralization (mucopolysaccharidosis Type IVA and Type II are used as examples). The lattice spacing of the hydroxyapatite phase, the crystallite size and aspect ratio, and the quantified preferred orientation of crystallites across whole intact tooth sections, have been determined using synchrotron microdiffraction.
Results: Significant differences in mineral crystallite orientation distribution of affected enamel have been observed compared to healthy mineralized tissue. The gradation of enamel crystal orientation seen in healthy tissue is absent in the affected enamel, indicating a continual disruption in the crystallite alignment during mineral formation.
Conclusions: This state of the art technique has the potential to provide a unique insight into the mechanisms leading to deranged enamel formation in a wide range of disease states.
Clinical relevance: Characterising crystal orientation patterns and geometry in health and following disruption can be a powerful tool in advancing our overall understanding of mechanisms leading to the tissue phenotypes seen clinically. Findings can be used to inform the appropriate dental management of these tissues and/or to investigate the influence of therapeutic interventions or external stressors which may impact on amelogenesis.

1. Introduction

Diseases associated with mineralization defects are frequently investigated using structural characterisation of affected hard tissues to complement an existing understanding of disease pathogenesis informed by cellular and molecular studies. A wide variety of techniques used to study bone and dental hard tissues include light and electron microscopy[1];

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0300-5712/$ – see front matter
http://dx.doi.org/10.1016/j.jdent.2012.08.020

atomic force microscopy[2,3]; X-ray microtomography[4,5]; X-ray diffractometry[6–8]; and increasingly synchrotron X-ray scattering.[9–11] In particular electron microscopy studies can show qualitatively hierarchical features on the microscopic length-scale such as prismatic and interprismatic structures of enamel revealing the variation in prismatic structure between species showing that enamel ultrastructure and function are closely linked to evolutionary development.[12] In particular, recent evaluation of Hunter–Schreger bands in human enamel reveal that these specific prismatic orientations have evolved to optimise resistance to fracture and wear over the lifetime of an individual.[13,14]

Uniquely, synchrotron X-ray microdiffraction can determine spatial distributions of basic crystallographic parameters of the hydroxyapatite (HA) phase within mineralized tissues. Characterising crystal orientation patterns and geometry in health and following disruption can therefore become a powerful tool advancing our overall understanding of mechanisms leading to phenotypic expression. Dental hard tissues are unique in terms of their accessibility for such analyses. Deciduous teeth exfoliate naturally and permanent teeth are frequently available following routine extraction. More importantly dental enamel is highly mineralized and it's unique hierarchical structure forms incrementally over extended time periods with individual teeth mineralizing in 4–5 years. Accordingly disruption in crystallographic features of dental enamel due to disease progression or therapeutic intervention can be closely correlated with event time points.

The spectrum metabolic disorders known as mucopolysaccharidosis (MPS) diseases have incidences reported to range from 1:50,000 to 1:250,000 births[15] and will be used as a case study to highlight the capabilities of the technique. In particular mucopolysaccharidosis Type IVA (MPS IVA), or Morquio Syndrome, has manifestations in primary and secondary dentition. MPS IVA, an autosomal recessive lysosomal storage disease,[16,17] is characterised by reduced activity of enzyme N-acetylgalactosamine 6-sulphatase (GALNS) encoded by a gene on human chromosome 16q24.3[18,19] which leads to intracellular accumulation of partially degraded glycosaminoglycans (GAGs) keratan sulphate and chondroitin 6-sulphate in connective tissue, the skeletal system and teeth.[20,21] Clinically it manifests after infancy and is associated with severe skeletal abnormalities, restrictive lung disease, impaired endurance, hearing impairment, and aortic valvular disease.[22] Enzyme therapies developed for MPS IVA are currently being investigated through clinical trials (NCT ID: NCT01242111 and NCT01275066), with the potential to revolutionise treatment for patients.

Basic dental histological investigations have demonstrated that MPS IVA enamel is abnormally thin and pitted[23] with increased porosity correlating to the striae of Retzius.[24] Electron microscopy has revealed an interstitial layer of amorphous material 3–4 μm thick at the amelodentinal junction (ADJ).[25] In MPS IVA it has been suggested that pathological accumulation of GAGs occurs in the lysosomes of secretory stage ameloblasts.[21] However, there is no consensus on whether the effects of impaired lysosomal pathway function result in disturbances in protein secretion; matrix mineralization; degradation processes of amelogenins; or a combination which lead to the enamel structural changes. 2D synchrotron X-ray diffraction across whole intact sections of dental enamel can provide important insights into the spatial distribution of HA crystallite orientation.[9,26] The aim of this study was to ally detailed analysis of physical characteristics of affected enamel in a system with a known, precise, underlying genetic lesion. We aim to demonstrate that the technique can not only give novel insight into the mechanistic understanding of the disease pathogenesis (in MPS), but also provide better understanding of basic processes of enamel biomineralization in health by relating known genetic defects to measured changes in crystallographic parameters.

2. Materials and methods

2.1. Specimen preparation

Tooth specimens were collected following ethical approval (UK National Research Ethics Service Reference 08/H1202/119) and consent at Birmingham Children's Hospital NHS Foundation Trust. Two deciduous maxillary incisors from different patients affected by MPS IVA; one affected by MPS II (with no previous reported effect on enamel formation); and one healthy control deciduous maxillary incisor were used. Each extracted tooth, stored in thymol-saline solution, was serially sectioned bucco-lingually using a 0.3 mm diamond blade cutter (Met Prep, Coventry, United Kingdom) then polished to 100 μm thick. A spatially equivalent 0.4 mm × 0.2 mm area, located 1 mm superior to the enamel–cementum junction was identified as a representative and comparable region of interest on each tooth section. An illustration highlighting the equivalent region of interest on each tooth section is given in Fig. 1. For synchrotron studies tooth sections were kept hydrated during measurement using a reservoir of thymol-saline solution. For scanning electron microscopy the sections were dehydrated in ethanol, etched with 35% orthophosphoric acid for 15 s, and stored in a desiccator prior to use.

2.2. Experimental procedure

Synchrotron X-ray diffraction was used to explore the texture (or preferred orientation) of enamel crystallites in intact tooth sections. Preferred orientation refers to the degree of crystallite alignment. For a polycrystalline, isotropic material, there is random orientation of crystallites averaging the Bragg scattering intensity uniformly around the Debye rings of 2D X-ray diffraction patterns. However, a high degree of crystalline anisotropy, such as in dental enamel, produces a change in intensity around the Debye ring of Bragg reflections in two-dimensions correlating to the degree of crystallite alignment or ordering (Fig. 2, inset A). This change in intensity around Debye rings from different regions of enamel was measured to quantify the spatial texture distribution, and therefore the enamel crystallite organisation as a function of position.

2D synchrotron X-ray diffraction experiments were carried out on the XMaS beamline (BM28) at the European Synchrotron Radiation Facility (ESRF). Performing experiments at central synchrotron radiation facilities through a peer-reviewed beamtime application process, means there are