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On the structure–property relationship of sound and hypomineralized enamel

Z.-H. Xie [a,b,*], E.K. Mahoney [c], N.M. Kilpatrick [d,e], M.V. Swain [b,f], M. Hoffman [a]

[a] *School of Materials Science and Engineering, University of New South Wales, NSW, Australia*
[b] *Biomaterials Research Unit, Faculty of Dentistry, University of Sydney, NSW, Australia*
[c] *Department of Dentistry, Hutt Valley District Health Board, Private Bag 31907, Lower Hutt, Wellington, New Zealand*
[d] *Oral Health Research, Murdoch Children's Research Institute, VIC, Australia*
[e] *Department of Paediatrics, University of Melbourne, VIC, Australia*
[f] *Oral Sciences, University of Otago, Dunedin, New Zealand*

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Abstract

Developmental defects in dental enamel pose significant clinical challenges which have highlighted our limited understanding of the structure and properties of this tissue. In this study, we first investigated the contact-size dependence of the physical properties of sound and hypomineralized enamel, and then examined the microstructure to establish a structural basis for their differing properties. Depth-sensing indentation tests were carried out over a wide range of peak loads in a direction perpendicular to the enamel prisms. Hypomineralized enamel demonstrated stronger penetration dependence for measured hardness and elastic modulus than sound enamel. The microstructure of sound and hypomineralized enamel was observed using field emission scanning electron microscopy and transmission electron microscopy with support of a focused ion beam milling system. Images of sound enamel showed barely distinguishable sheath regions with minimal organic presence. In contrast, hypomineralized enamel showed thicker sheath structures surrounding the prisms and higher levels of organic content within both the prisms and the sheath regions. It is argued that the higher organic content within prism structure was responsible for an initial lower hardness and elastic modulus of hypomineralized enamel under low-load indentation. As the indentation depth increased, the thicker organic-rich sheath regions played a more important role in reducing the mechanical properties of the hypomineralized enamel. On the basis of Spears finite element model [Spears IR. A three-dimensional finite element model of prismatic enamel: a re-appraisal of the data on the Young's modulus of enamel. J Dental Res 1997; 76(10):1690–97], elastic moduli of sound and hypomineralized enamel were predicted, which matched experimental results.

Keywords: Enamel; Hypomineralization; Elastic modulus and hardness; Focused ion beam milling; Electron microscopy

1. Introduction

Isolated developmental enamel defects are commonly seen in first permanent molar teeth, with up to 19.3% of children affected [2]. These enamel defects are usually a combination of hypoplasia and hypomineralization [3]. Hypoplasia is caused by a disruption to the ameloblasts during matrix secretion and results in a quantitative defect of the enamel. If the disruption occurs during either the calcification or maturation phase of amelogenesis, there will be a qualitative hypomineralized defect in the enamel. Clinically, hypomineralized defects present as white-yellow or yellow-brown demarcated opacities which vary greatly in their size, colour and shape. However, the affected enamel is consistently soft and chips away easily under masticatory forces as soon as the teeth erupt. These compromised teeth present challenges, with a large number of restorations

* Corresponding author. Address: School of Materials Science and Engineering, University of New South Wales, NSW, Australia. Tel.: +61 402 723 248; fax: +61 2 9385 5956.
E-mail address: zhxie@materials.unsw.edu.au (Z.-H. Xie).

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placed in these teeth failing, leading to the extraction of the tooth [4]. Greater knowledge and understanding of the structure, composition and mechanical properties of hypomineralized enamel may explain the unacceptably poor prognosis associated with these teeth and inform more appropriate treatment strategies in the future.

The structural units of enamel are prisms or rod-like structures which radiate from the dentino-enamel junction perpendicularly to the surface, with an average diameter of 4×5 m. Neighbouring enamel prisms are joined to each other by organic-rich sheaths. Within the prisms hydroxyapatite crystals constitute the dominant mineral phase fused by nanometer thick protein layers. An understanding of the mechanical properties of enamel is very important to evaluate the behaviour of hypomineralized enamel during mastication and assist development of restorative materials and techniques. The hardness and elastic modulus of sound enamel has been shown to range from 4 to 6 and from 40 to 100 GPa, respectively, using a nanoindentation approach [5–8]. The wide range is due to a variety of indenter types, indentation orientations relative to the enamel prisms, distance of indentation from the enamel/dentine junction and a contact-size effect [7]. A recent nanoindentation study on the plane parallel to the prism direction has shown that the hardness and modulus of hypomineralized enamel are significantly lower than those of sound enamel, with measured values of 0.53 ± 0.31 and 14.49 ± 7.56 GPa, respectively [9]. Attempts to determine the cause of this significant reduction have so far been inconclusive [10]. X-ray diffraction analysis of hypomineralized enamel revealed an apatite phase with no detectable change in the crystallinity compared with sound enamel. Neither were additional ions or phases found in the defective structure. Furthermore, the use of energy dispersive spectroscopy in a scanning electron microscope (SEM) revealed no differences in the Ca/P ratio, suggesting that neither the mineral phase is altered nor significant substitution has occurred. A slight difference in grey-level intensity has been detected under back-scattering mode in the SEM suggestive of a slight reduction in mineral content [10]. Similarly, a reduction in mineral density of hypomineralized enamel of $\sim$2.0 g cm^{-3} vs. $\sim$2.60 g cm^{-3} for sound enamel has been reported using microcomputer tomography (microCT) imaging [11].

It is evident, therefore, that the dramatic reductions in both hardness and elastic modulus of hypomineralized compared with sound enamel cannot be fully explained by changes in crystal structure, chemistry and/or mineral density. In this work, we seek to further elucidate the reasons for this reduction in mechanical properties. We explore the multi-scale structure of enamel by undertaking nanoindentation at varying loads, hence measuring the mechanical properties, both within the prisms and the entire prism structure. We then undertake advanced electron microscopy analysis to elucidate the changes in microstructure and architecture associated with compromised enamel.

2. Materials and methods

2.1. Sample preparation

Ethical approval and patient informed consent was gained from Western Sydney Area Health Service Ethics Committee for the collection of teeth for this study. The demanding nature of subsurface cross-sectional transmission electron microscopy (TEM) specimen preparation limited the number of teeth that can be used in this investigation. Therefore, great attention was paid in the beginning when choosing the teeth for this experiment. Our earlier work [9,10] clarified the distinct mechanical property (outlined later) and aesthetic differences between normal and hypomineralized dental enamel. Two types of teeth, i.e. a first permanent molar extracted with a significant developmental defect in the enamel and a sound control tooth (a first premolar extracted for orthodontic reasons), were used in this study. The test tooth investigated with a significant developmental defect in enamel was qualified in accordance with the clinical definition of molar–incisor hypomineralization [12]. The subsequent mechanical testing also confirmed its nature in comparison to the sound control tooth. Upon extraction, both teeth were placed into Hanks' balanced salt solution (Sigma–Aldrich Co., St. Louis, MO) with thymol crystals added to inhibit bacterial growth and stored at 4 °C.

The initial preparation of the specimens has been described in detail in an earlier paper [8]. Each tooth was encased in cold cured epoxy resin and sectioned with two parallel cuts through the centre of the lesion in the mesial–distal axial planes using a water-cooled diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL). The cutting directions were selected so that prisms were parallel with the cut surface. The resulting slice, approximately 2 mm thick, was polished on one cut surface with successively finer grade silicon carbide paper and finally with 9 and 1 μm diamond suspension. Once prepared, specimens were kept fully hydrated in Hanks' balanced salt solution with the addition of thymol crystals.

2.2. Mechanical characterization

In order to measure hardness (H) and elastic modulus (E) of enamels, indentation experiments were conducted using a depth-sensing indentation system (Ultra-Micro Indentation System 2000, Sydney, Australia) with a Berkovich indenter (Synton, Switzerland). The area function of the indenter was calibrated using standard fused silica. Both the calibration procedure and the method used for indentation hardness and modulus measurement are based upon a widely adopted method [13]. The indentation results were determined with known values of elastic modulus and Poisson's ratio for diamond tip (1070 GPa and 0.07, respectively) along with Poisson's ratio of enamel of 0.3 [14].