Acta Biomaterialia 10 (2014) 5169–5177

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Compositional, structural and mechanical comparisons of normal enamel and hypomaturation enamel

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ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 29 July 2014
Accepted 19 August 2014
Available online 27 August 2014

Keywords:
Enamel
Hypomaturation
Composition
Structure
Mechanical property

ABSTRACT

Hypomaturation amelogenesis imperfecta is a hereditary disorder of the enamel that severely influences the function, aesthetics and psychosocial well-being of patients. In this study, we performed a thorough comparison of normal and hypomaturation enamel through a series of systematical tests on human permanent molars to understand the biomineralization process during pathological amelogenesis. The results of microcomputed tomography, scanning electron microscopy, Fourier transform infrared, Raman spectroscopy, microzone X-ray diffraction, thermal gravimetric analysis, energy diffraction spectrum and Vickers microhardness testing together show dramatic contrasts between hypomaturation enamel and normal enamel in terms of their hierarchical structures, spectral features, crystallographic characteristics, thermodynamic behavior, mineral distribution and mechanical property. Our current study highlights the importance of the organic matrix during the amelogenesis process. It is found that the retention of the organic matrix will influence the quantity, quality and distribution of mineral crystals, which will further demolish the hierarchical architecture of the enamel and affect the related mechanical property. In addition, the high carbonate content in hypomaturation enamel influences the crystallinity, crystal size and solubility of hydroxyapatite crystals. These results deepen our understanding of hypomaturation enamel biomineralization during amelogenesis, explain the clinical manifestations of hypomaturation enamel, provide fundamental evidence to help dentists choose optimal therapeutic strategies and lead to improved biofabrication and gene therapies.

1. Introduction

Enamel is the outer structure of the tooth, which envelops the whole crown and protects the underlying dentin and pulp against mechanical wear, acidic erosion and bacteria attack in our daily lives [1–4]. Unlike other biominerals, however, enamel is a non-living tissue and has no capacity to regenerate, self-repair or remodel [5,6]. As a result, the structural and compositional properties of enamel are extremely important to its inherent biomechanical functions.

Previous findings have contributed greatly to our knowledge about the structure of normal enamel [7–10]. They reveal that is has an intricate organic–inorganic hybrid organization with hierarchical levels. First, needle-like hydroxyapatite (HAP) crystals pack tightly into groups and the organic matrix binds these crystals together [11,12]. The crystals are then organized into the base structural unit, the enamel prism, in a species-specific manner. Lastly, prisms and interprism structures assemble into prism bands across the thickness of the enamel layer. These well-organized structures endow normal enamel with its superior strength and toughness [9,13,14]. However, there are some pathological forms of enamel that affect patients' day-to-day lives.

Hypomaturation amelogenesis imperfecta is a hereditary disorder of enamel [15–18]. The afflicted enamel reveals a wide spectrum of clinical manifestations, including a discolored appearance, a similar radiodensity to dentin, a soft texture and high vulnerability to dental caries [19–21]. As a result, hypomaturation enamel severely influences the function, aesthetics and psychosocial well-being of patients. However, owing to the scarcity of available enamel samples and/or the lack of appropriate technologies, hypomaturation enamel is still poorly investigated. To the best of our knowledge, a limited number of studies have simply observed the structure of hypomaturation enamel and evaluated its elemental and protein content [22,23]. Detailed molecular, crystallographic and compositional knowledge of hypomaturation enamel

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http://dx.doi.org/10.1016/j.actbio.2014.08.023
1742-7061/

is still deficient and no previous study has systematically evaluated the structural organization of hypomaturation enamel at different hierarchical levels.

The compositional and structural properties of enamel are in fact very important. More compositional comparisons between hypomaturation enamel and normal enamel would better reveal how organic and inorganic matter affects the final structure of enamel after the biomineralization process. On the basis of structural information, it is helpful for dentists to explain the mechanical property and the related clinical manifestations of hypomaturation enamel to patients and then choose an optimal therapeutic strategy. More importantly, hypomaturation enamel is a powerful model of natural biominerals. Compositional and structural contrasts between hypomaturation enamel and normal enamel can help scientists understand the genetic processes during enamel biofabrication or synthesize novel biomimetic materials for enamel tissue engineering. Herein, the overall objective is to use a series of techniques to compare the compositional, structural and mechanical properties of permanent hypomaturation enamel and normal enamel.

2. Materials and methods

2.1. Tooth collection

Under a protocol approved by the local Ethics Committee of the School and Hospital of Stomatology, Wuhan University, People's Republic of China, three impacted third molars affected with autosomal recessive pigmented hypomaturation amelogenesis imperfecta (Fig. 1) were obtained with informed consent from a 20-year-old Han Chinese female from Wuhan City of Hubei Province and a 24-year-old Han Chinese male from Xiangfan City of Hubei Province (two from the female and one from the male). In order to minimize the variation in tooth location between the hypomaturation and normal enamel, three impacted third molars from two unaffected adults, ages 21 and 24 years, were chosen as controls. All teeth were cleaned thoroughly and then stored in 0.2% thymol solution at 4 °C until use [24–26].

2.2. Study design

For comprehensive analysis of the enamel, component identification, quantity and quality analyses of components and local mineral distribution were investigated by complementary use of Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, microzone X-ray diffraction (microzone XRD), thermal gravimetric analysis (TGA) and energy-dispersive spectroscopy (EDS) measurements. The hierarchical structures for both enamel types were evaluated through the use of microcomputerized tomography (micro-CT) and scanning electron microscopy (SEM) measurements. Further, the structure-related mechanical property was probed by Vickers microhardness measurement. Due to the limited number of samples and the use of a variety of techniques, measurements were performed according to a particular sequence as shown in Fig. 2.

2.3. Micro-CT

The roots of the collected teeth were separated from their crowns at the cemento-enamel junction with a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling. Hypomaturation and normal tooth crowns were scanned by a micro-CT imaging system (Micro-CT 50, Scanco Medical, Basersdorf, Switzerland). Samples were placed in a custom-made holder to ensure that the long axis of the enamel were oriented perpendicular to the axis of the X-ray beam. Scanning was performed at 70 kV and 200 mA in high-resolution mode, with a 1024 reconstruction matrix and 400 ms integration time. Collected digitalized data was used to reconstruct the 3-D images of samples by the built-in software of the micro-CT.

After the micro-CT test, each tooth was longitudinally sectioned into two halves (Fig. 2). The lingual halves were used for SEM/EDS observation and the buccal halves were used for microzone XRD, Raman spectroscopy, Vickers microhardness measurements, FTIR and TGA.

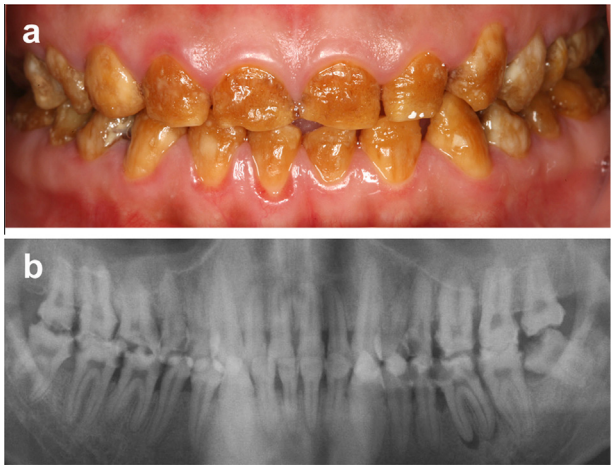

Fig. 1. Clinical and radiographic examination of hypomaturation enamel. (a) Clinical appearance of hypomaturation enamel. (b) Panoramic radiograph of a patient afflicted with hypomaturation enamel.

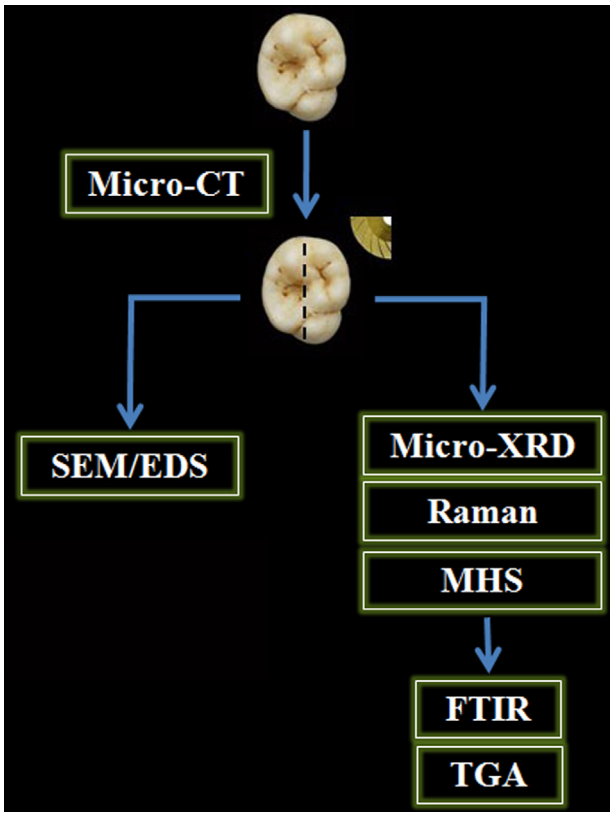

Fig. 2. Flow diagram of the experimental process. MHS stands for Vickers microhardness test.