Study of developmental enamel defects of permanent teeth by atomic force microscopy

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J. O. Turner was the first who described the developmental defect of permanent tooth caused by periapical inflammation of temporary predecessor. This disorder is most common in the upper front teeth and premolars. These disorders are collectively called developmental defects of enamel (DDE). They are visible deviations from the normal translucent enamel resulting from enamel organ dysfunction. According to the macroscopic appearance we distinguish three types: enamel hypoplasia, diffuse enamel opacity, demarcated enamel opacity. The combination of types of enamel defects found in one tooth is also quite common. Results from studies at sheep and studies of macroscopic appearance, hardness values and SEM examination of human enamel defects of permanent teeth support this division and demonstrate that the pathogenesis of each type is different. DDE was imaged by atomic force microscopy Bruker Bioscope Catalyst using Peak force Quantitative NanoMechanical measurement for imaging and mechanical characterization. Differences between enamel of health part of tooth and DDE were studied.

Keywords Developmental defects of enamel; DDE index; Permanent teeth

1. Introduction

In 1906 J.O. Turner described a development disorder of permanent tooth caused by inflammation of periapical temporary predecessor [16]. This disorder is most common by the upper anterior teeth and premolars because of the close anatomical relationship between the roots of deciduous teeth and dental germs [17]. It manifests as discolorations, opacities and enamel hypoplastic lesions on permanent teeth. The most common causes of inflammation of deciduous teeth is Early Childhood Caries (ECC) and its complications. The relationship between caries in the deciduous dentition and finding opacities and hypoplasia demonstrated Lo et al. in the study of Chinese children in nonfluoridated area [9].

As a result of high prevalence of Early Childhood Caries in the pediatric population we can also expect a higher incidence of Developmental Defects of Enamel (DDE) at permanent teeth. It entails an aesthetic handicap and associated complications DDE such as failure of eruption of permanent teeth, calcifications in pulp cavity or more frequent development of periodontitis of DDE suffering teeth [9, 1]. A DDE index is used to divide enamel defects into one of three main types [15]:

1. Hypoplasia, characterized by a reduced thickness of enamel. The absence of enamel may be partial or complete. The extent vary, resulting in pit(s), groove(s), or larger areas of missing enamel.
2. Demarcated opacities, have an abnormality in the translucency but no in the thickness of enamel. These lesions have a clear boundary separating abnormal and normal enamel. Variations occur in their color, which ranges from white to cream, yellow, and in degree of translucency.
3. Diffuse opacities are alterations in translucency and have no change in enamel thickness. They do not have a clearly defined margin. Fluoride-induced lesions are found in this type.

Dental enamel is the hardest substance of human body. The ultrastructure has been widely investigated, showing that the inorganic components are distributed in the form of rods or prisms, which are composed of hydroxideapatite crystals [6, 12].

The aim of this research is to analyze the differences between the surface of healthy enamel and DDE area with using an atomic force microscope.

2. Material and Methods

2.1 Material and Instruments

In our experiments we used a human tooth with DDE-demarcated opacity, as a biological material. It was the permanent incisor of 7 years old girl, which was lost due to avulsion. In medical history of the patient was ECC with extraction of deciduous incisors for periodontitis. On the labial area were two bordered creamy white opaque irregular opacities. Measurements were carried out on AFM Bioscope Catalyst (Bruker USA) and with transmission microscope IX81.
(Olympus, Japan), using silicon tip on nitride lever ScanAsyst Air tip (Bruker, USA). Tooth was put on the adhesive tape.

![Image](image1.jpg)

**Figure 1** The investigated tooth with two demarcated opacities. In our research we examined the bigger one in the middle.

2.2 Sample preparation

Freshly extracted permanent incisor was cleaned and stored in 10% formaldehyde solution in. It was cut into 3 mm thick disc of hand grinding wheel. This disc was the whole labial part of tooth. It was not modified, only dried.

2.3 AFM imaging

The tooth was imaged with a scan rate of 0.1 Hz. We used ScanAsyst Air tip with a resonant frequency of 50 - 90 kHz and a force constant of 0.4 N.m⁻¹. AFM surface images were acquired in a Peak Force QNM imaging mode. All images were processed by Nanoscope Analysis (Bruker, USA). After recording scan, the tip was placed on the identified place of tooth (DDE, enamel) and force curves were measured in each point (pixel) of scan. The modulus of elasticity (stiffness) was obtained by fitting the retract curve using the Derjaguin, Muller, Toropov (DMT) model [5].

3. Results and Discussion

Enamel is the hardest substance of the body because of its high mineral content (92-96%). However, it has relatively low resistance to fracture, which is attenuated by the particular arrangement of the inorganic components distributed in the form of rods or prisms [7]. The rods stand upright on the surface of the dentine and run through the whole thickness of the enamel layer [6]. In our research we investigated with using the AFM microscope the surface of healthy enamel and enamel with DDE-demarcated opacity. We compared the modulus of elasticity and the altitude profile of healthy and modified enamel. The first pair of images (Figure 2) shows a comparison of the surface of healthy enamel to the surface of DDE in the magnification 100 × 100 µm. There is clearly shown the difference in structure of hard dental tissue. The surface of DDE is rougher, wrinkled and irregular. The same situation but at higher magnification shows following series of images (Figure 3 and 4).

![Image](image2.jpg)

**Figure 2** The 3D topography images of enamel (left) and DDE (right) part of tooth in 100 × 100 µm scan. The scan was obtained by Peak Force QNM imaging mode. Parameters of the pictures are: resolution 256 pixels scan rate 0.1 Hz. Height of enamel structure is between 0 (dark fields) – 4.4 µm (light fields), height of DDE is 0 – 5.3 µm.
Significant height differences of surface structures normal enamel and DDE can be seen on the graphs 1 – 3. In some places inequality amounts to 2 µm of high. Surface structure corresponds with the morphology of mammalian enamel. Mammals have in the most superficial region of enamel a very thin layer of apatite crystals oriented perpendicular to the surface, so called aprismatic enamel [2, 4, 8].

**Figure 3** The 3D topography images of enamel (left) and DDE (right) part of tooth in 10 × 10 µm scan. The scan was obtained by Peak Force QNM imaging mode. Parameters of the pictures are: resolution 256 pixels, scan rate 0.1 Hz. Height of enamel structure is between 0 (dark fields) – 300 nm (light fields), height of DDE is 0 – 1.7 µm.

**Graph 1** The line profile of enamel and DDE samples in line number 220.

**Graph 2** The line profile of enamel and DDE samples in line number 215.
The organization of crystals is complicated, they are packed in very close contact and create clusters. The projections of clusters on the surface in image Figure 4 look like aggregated globular particles of varying sizes. In our observations of the shapes of particles in aprismatic enamel we find no geometrical symmetry. The smaller particles in images in work of Farina et al. was in range 75nm and may represent cross sections of individual apatite crystals. Most of crystalittes are closely attached to each other so particles in images present more than one enamel crystal [6]. Better imaging of the structure of enamel prisms is obtained by cutting the DDE area parallel to the axial axis. We can study the course of prisms from the dentinoenamel junction to the enamel surface. This we plan to do as a next part of our research.

![Image of crystal clusters](image1)

**Figure 4** The 3D topography images of enamel (left) and DDE (right) part of tooth in $1 \times 1$ µm scan. The scan was obtained by Peak Force QNM imaging mode. Parameters of the pictures are: resolution 256 pixels, scan rate 0.1 Hz. Height of enamel structure is between 0 (dark fields) – 55 nm (light fields), height of DDE is 0 – 260 nm.

![Graph showing line profile of enamel and DDE samples](image2)

**Graph 3** The line profile of enamel and DDE samples in line number 135.

The DMT modulus (elasticity) is imaged in Figure 4. The reduced elastic modulus $E^*$ was obtained by fitting the retract curve using the Derjaguin, Muller, Toropov (DMT) model [5]:

$$F = \frac{4}{3} E^* \sqrt{Rd^3} + F_{adh} \ldots (1)$$

where $F$ is the force, $R$ is the tip radius, $d$ is the separation, $F_{adh}$ is the adhesion force, and $E^*$ is the reduced elastic modulus. By assuming infinite elastic modulus for the tip ($E_{tip}$) and knowing the Poisson’s ratio of the sample $\nu_s$, the Young’s modulus for the sample ($E_s$) can be calculated using following equations [10]:

$$E^* = \left( \frac{1-\nu_s^2}{E_s} + \frac{1-\nu_{tip}^2}{E_{tip}} \right) E_{tip} \ldots (2)$$

DMT modulus determined the elasticity of enamel and its modification. From the perspective of flexibility, the enamel is compact structure without significant changes in elasticity of the structure (see Figure 4). Conversely, DDE
The enamel is an extracellular product of ectodermal cells ameloblasts with 3 distinguishing features- apatite crystallites, organic matrix with collagen, few remnants of organic matrix after complete of mineralization.

Systemic or local disorders of ameloblasts during their active involvement can result in developmental defects of enamel [14]. Although there are only a few types of enamel defects, Small and Murray [13] and Pindborg [11] suggested more than 50 etiological factors of DDE in addition to fluoride, which can be divided into systemic and local factors. Most studies have focused on enamel defects associated with systemic factors [9]. Our research deals with disorders of dental hard tissues formed in connection with local irritation. In this case it was an inflammation resulting from ECC development what mutilate the germin of permanent incisor. Nearly all the visible defects of the enamel of human teeth can be classified according to macroscopic appearance into one of three types (DDE index) - hypoplasia, diffuse and demarcated opacity [14, 3]. The combination of multiple types of enamel defects found in one tooth is also quite common. Results from studies of macroscopic appearance, hardness values and SEM examination of human enamel defects of permanent teeth support this division and demonstrate the different pathogenesis of each type [14].

Hardness values in Table 1 show that in our research is the DDE region much harder than healthy enamel. The work of Suckling [15] conversely evaluates the defect Demarcated opacity as much softer. This discrepancy may be caused by a completely different technique of hardness measure - Suckling determined hardness of DDE on the cut from the surface to the dentino-enamel junction, our research focused on determining the hardness values from the surface layer of enamel.

The atomic force microscopy was used to study enamel surface with aim to compare the pattern of particle distribution in healthy enamel and in DDE. AFM gives high contrast, high-resolution images and is an important tool as a source of structural information.

### Table 1 Elasticity modulus of teeth surface in arbitrary unit (Arb)

<table>
<thead>
<tr>
<th>Tooth surface magnification</th>
<th>Elasticity modulus DMT (Arb)</th>
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<tbody>
<tr>
<td>DDE 1x</td>
<td>188.4</td>
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<tr>
<td>DDE 10x</td>
<td>85.8</td>
</tr>
<tr>
<td>DDE 100x</td>
<td>49.5</td>
</tr>
<tr>
<td>Enamel 1x</td>
<td>22</td>
</tr>
<tr>
<td>Enamel 10x</td>
<td>14.9</td>
</tr>
<tr>
<td>Enamel 100x</td>
<td>10.6</td>
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References