Chemical Composition and Crystalline Structure of Hypoplastic Primary Dental Enamel

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Abstract
The present study, reports the chemical composition of hypoplastic primary teeth examined by electron probe microanalyzer comparing with the normal part in the same tooth; and reports the X-ray diffraction results of the hypoplastic dental enamel and the normal dental enamel, and comparing the crystalline structure between the two dental enamel together with that of the synthetic hydroxyapatite. The aim of this study was to find out any change of crystalline structure. Two exfoliated hypoplastic deciduous anterior teeth were used for the electron probe study. Each tooth was sliced into two parts along the defect lesion border in a labio-lingual direction. The normal part was used as control. Determinations of weight percentage (wt%) per mass volume were made in continuous scan for P, Al, Mg, Ca, Mn, Fe, Zn, Sr, Na, K and F, from the enamel edge of hypoplastic bottom to dentin-enamel junction. Four exfoliated hypoplastic deciduous anterior teeth and eight exfoliated normal deciduous anterior teeth were used for the X-ray diffraction study. We found by X-ray diffraction that the length of the a-axis of enamel crystallite and the d-spacing (corresponding 300) were increased in the defective enamel. These could be associated with the increased content of magnesium detected by electron probe. The present study demonstrated that there were both quantity and quality changes in the enamel hypoplasia lesion, which may increase the susceptibility of the defective teeth to caries.

Introduction
The chemical composition and the crystalline structure of dental enamel are close to those of mineral hydroxyapatite (HA[5]). Relatively small differences in the dimensions of a unit cell, the degree of crystallinity and the elemental structure have been linked to substituents in the lattice of enamel(1, 2). Although these impurities, which include Na, K, Mg, CO3, Sr (strontium) and F, constitute only a fraction of the enamel, they are particularly effective at modifying or regulating its response to environmental agents(3). As reported by Patel and Brown(4), impurities in the lattice are largely responsible for the distinctly enhanced acid-solubility of human enamel compared to that of hydroxyapatite.

Enamel hypoplasia is a morphological defect as a result of metabolic disturbance during enamel development and calcification. The studies on chemical composition and crystalline structure of enamel hypoplasia not only help to understand the essence of enamel hypoplasia, but also to probe into the pathogenesis of this disease in a deepgoing way. Meanwhile, we can also probe into the relationship between hypoplasia teeth and their susceptibility to dental caries with information of chemical composition and crystalline structure. With regard to the study of crystalline structure of dental enamel, researchers have focused their attention on the permanent teeth. Few studies on crystalline structure of hypoplastic primary teeth could be found in the literature.

Gao et al. (5,6) have studied crystalline structure of de- and remineralization of dental enamel by X-ray diffraction. They found changes in crystalline structure during de- and remineralization processes. The present study, firstly, analyzed chemical composition of hypoplastic primary teeth by electron probe analyzer and compared the hypoplastic part with the normal part in the same tooth; secondly, by X-ray diffraction analyzer, studied the hypoplastic dental enamel and the normal dental enamel, and compared the crystalline structure between different dental enamel and with that of the hydroxyapatite. The aim was to find out
any change of crystalline structure. Combining with the clinical materials, we could further understand the essence of enamel hypoplasia, and probed into the relationship between hypoplastic teeth and their susceptibility to dental caries.

**Materials and Methods**

**Sample**

Six exfoliated hypoplastic deciduous anterior teeth, which were collected from the children only with histories of low birth weight and premature birth. Two were used for the electron probe study and four were used for X-ray diffraction. Eight exfoliated normal deciduous anterior teeth, in which each two were collected from the children with histories of only low birth weight, or only premature birth, both low birth weight and premature birth, full term and normal birth weight, respectively. These teeth were stored in normal saline with 2% thymol at 4°C.

**Electron Probe Microanalysis**

Each tooth was sliced into the mesial and distal parts along the defect lesion border in a labio-lingual direction. The normal part was used as control. Sections were polished with diamond powder and then coated in a vacuum evaporator with a uniform layer of carbon. The teeth were analyzed with CAMECA SX 50 electron probe analyzer (15.0 kV, 20nA). The standards used were fluorapatite for P and Ca, oxide for Al and Mg, rhodonite for Mn, ferric oxide for Fe, zinc oxide for Zn, strontium sulfate for Sr, albite for Na, potash feldspar for K, fluorite for F. Determinations of weight percentage (wt%) per mass volume were made in point scan for P, Al, Mg, Ca, Mn, Fe, Zn, Sr, Na, K and F, from the enamel edge of hypoplastic bottom to dentin-enamel junction, and these elements of each point were analysed simultaneously. These elements of the normal part in the same tooth were analysed in the same mode. The interval for the former three points in the section was 20 μm, the interval for the rest points was 50 μm.

Student-t test was applied to reveal significance of differences.

**X-ray powder diffraction**

**Preparation of samples**

Following the previous method(6), enamel powder was obtained by cutting off the remnant root, separating the normal part and the lesion part (only for the hypoplastic primary teeth), grinding off dentin from the inside of tooth crowns with a high speed dental bar, and then crushing the crown pieces with a glass mortar and pestle.

**Sample**

Sample 1 was named as the synthetic HA (E. Merck, HA, lot 123F 429896 after 950°C, F.W. 1004), Sample 2 was from the lesion parts of the four hypoplastic teeth, Sample 3 was from the normal parts of the four hypoplastic teeth, Sample 4 was from two normal teeth of the children with histories of only low birth weight, Sample 5 was from two normal teeth of the children with histories of only premature birth. Sample 6 was from two normal teeth of the children with histories of both low birth weight and premature birth, Sample 7 was from two normal teeth of the children with histories of full term and normal birth weight.

**Experimental conditions**

Samples were analyzed with XD-DI X-ray diffraction analyzer (Shimadzu Co., Japan) at 40kV, 40 mA. Cu Kα, λ=1.5405ÅThe unit cell dimensions were calculated by the following formula using the diffraction angles of 002 and 300 peak.

\[
\sin^2\theta_{hk} = (\lambda^2/3a^2)(h^2+k^2+2hk)+((\lambda^2/4c^2))l^2
\]

**Results**

**Composition of defected enamel compared with sound enamel**

Table 1 shows that mean and standard deviation of weight percentage (wt %) for Ca, P and Mg in the hypoplastic parts and normal parts of primary teeth. Compared with the normal part, the Ca wt% of the hypoplastic part was significantly lower (t=2.03, p<0.05), the Mg wt% significantly higher (t=2.62, p<0.05), the wt% of P (t=0.58, p>0.05) and other composition no change (Tables 2-4).

2. Crystallinity shown by X-ray Powder Diffraction

Figs.1-4 show the X-ray diffraction patterns of samples 1-4 (the X-ray diffraction patterns of samples 5-7 are the same as that of sample 4). The patterns of enamel were
Table 1. Composition of hypoplastic enamel compared with sound enamel (wt %)

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th></th>
<th>P</th>
<th></th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{X})</td>
<td>SD</td>
<td>(\bar{X})</td>
<td>SD</td>
<td>(\bar{X})</td>
</tr>
<tr>
<td>Hypoplastic part (n1=24)</td>
<td>37.238</td>
<td>1.137</td>
<td>17.960</td>
<td>0.542</td>
<td>0.399</td>
</tr>
<tr>
<td>Normal part (n2=36)</td>
<td>37.742</td>
<td>0.795</td>
<td>17.886</td>
<td>0.373</td>
<td>0.295</td>
</tr>
</tbody>
</table>

\(\bar{X}\), mean value; SD, standard deviation
n, the number of the points analyzed by electron probe;
n1, the hypoplastic part; n2, the normal part

Discussion

As showed in Figs. 1-4, the x-ray diffraction patterns of enamel powder (samples 2-7) were basically comparable with that of the synthetic hydroxyapatite, but there were some differences in the diffraction angle, the lengths of the \(a\)-axis and the \(c\)-axis, the \(a/c\) ratio and the distance of lattice plane (Tables 5, 6 and 7). Those included decreased diffraction angles of 002 peak and 300 peak, increased lengths of the \(a\)-axis and the \(c\)-axis, increased \(a/c\) ratio and increased distance of lattice plane (Tables 5, 6 and 7). The differences may be linked to impurities in the lattice of enamel(1, 2). The bigger unit cell size and distance of lattice plane indicated the lower degree of crystallinity(7, 8).

Fig. 1. The X-ray diffraction pattern of Sample 1.

Fig. 2. The X-ray diffraction pattern of Sample 2.

Fig. 3. The X-ray diffraction pattern of Sample 3.

Fig. 4. The X-ray diffraction pattern of Sample 4.
Table 2. Composition of hypoplastic enamel compared with sound enamel (wt %)

<table>
<thead>
<tr>
<th></th>
<th>Zn</th>
<th>Sr</th>
<th>Al</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>SD</td>
<td>$\bar{X}$</td>
</tr>
<tr>
<td>Hypoplastic part ($n_1=24$)</td>
<td>0.038</td>
<td>0.076</td>
<td>0.019</td>
</tr>
<tr>
<td>Normal part ($n_2=36$)</td>
<td>0.048</td>
<td>0.051</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Table 3. Composition of hypoplastic enamel compared with sound enamel (wt %)

<table>
<thead>
<tr>
<th></th>
<th>Mn</th>
<th>Fe</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>SD</td>
<td>$\bar{X}$</td>
</tr>
<tr>
<td>Hypoplastic part ($n_1=24$)</td>
<td>0.009</td>
<td>0.015</td>
<td>0.054</td>
</tr>
<tr>
<td>Normal part ($n_2=36$)</td>
<td>0.009</td>
<td>0.013</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Table 4. Composition of hypoplastic enamel compared with sound enamel (wt %)

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>F</th>
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<tbody>
<tr>
<td></td>
<td>$\bar{X}$</td>
<td>SD</td>
</tr>
<tr>
<td>Hypoplastic part ($n_1=24$)</td>
<td>0.052</td>
<td>0.027</td>
</tr>
<tr>
<td>Normal part ($n_2=36$)</td>
<td>0.065</td>
<td>0.039</td>
</tr>
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</table>

Table 5. The diffraction angles ($2\theta$) of the 002 peak and the 300 peak for samples 1-7

<table>
<thead>
<tr>
<th>hkl</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>32.887</td>
<td>32.511</td>
<td>32.630</td>
<td>32.636</td>
<td>32.636</td>
<td>32.636</td>
<td>32.636</td>
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Table 6. The lengths (Å) of the a-axis and the c-axis of a unit cell and the a/c ratio.

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<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>a/c</td>
<td>1.364</td>
<td>1.373</td>
<td>1.367</td>
<td>1.367</td>
<td>1.367</td>
<td>1.367</td>
<td>1.367</td>
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</table>

Table 7. The distance (Å) of lattice plane (corresponding 002 and 300)

<table>
<thead>
<tr>
<th>hkl</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>2.721</td>
<td>2.752</td>
<td>2.742</td>
<td>2.742</td>
<td>2.742</td>
<td>2.742</td>
<td>2.742</td>
</tr>
</tbody>
</table>
Compared with the synthetic HA, all enamel powder (samples 2-7) demonstrated bigger unit cell size and distance of lattice plane, which reflected the decreased degree of crystallinity.

The diffraction angle, the lengths of $a$-axis and $c$-axis, the $a/c$ ratio and the distance of lattice plane showed completely no difference in samples 4-7 (Tables 5, 6 and 7), which indicated that the crystalline structure and the degree of crystallinity of the normal primary dental enamel from children with histories of low birth weight and premature birth were the same as those of the normal primary dental enamel from children with histories of full term and normal birth weight. Although compared with samples 4-7, the above-mentioned parameters of sample 3 had little differences, these parameters were almost the same as those of samples 4-7 (Tables 5, 6 and 7). For this reason, the crystalline structure and the degree of crystallinity of the enamel of the normal part in the hypoplastic teeth were no difference from those of the enamel of the normal primary teeth.

The most important finding of the present study was made in sample 2 (the hypoplastic enamel powder). Compared with other enamel powder, the length of the $a$-axis of enamel crystallite increased, while the length of the $c$-axis was not affected. Meanwhile, the distance of lattice plane (corresponding 300) also increased. This suggests a change of crystalline structure and the crystallinity of the defective enamel. Any ions substitution in HA was accompanied by smaller or larger changes in the unit cell dimensions, the length of the $a$-axis usually being more affected than the length of the $c$-axis(9).

The electron probe study showed that the defective enamel contained higher Mg, lower Ca and unchanged P (Table 1). Mg can disturb the crystallization of the HA, result with characteristics of poorly crystallized enamel, and a delay in enamel mineralization(10-13). From this respect, we assumed that the increased length of the $a$-axis, the increased distance of lattice plane (corresponding 300) and the decreased crystallinity of the defective enamel should be due to the substitution of Mg for Ca.

The bigger distance of lattice plane and unit cell size implied that the lattice energy, and thus the resistance to dissolution were lower(7). The increased Mg can increase the acid-solubility of the enamel, making the enamel much easily affected by caries(14-17). Hence, the acid-solubility and the susceptibility to dental caries would increase in the hypoplastic enamel, which had an important clinical significance. By a clinical study we have found the prevalence of enamel hypoplasia could be significantly interrelated to the prevalence of dental caries in particular teeth(18,19).

**Conclusion**

To sum up, the present study demonstrates that the length of the $a$-axis of enamel crystallite and the distance of lattice plane (corresponding 300) were increased in the defective enamel. Those resulted in the decrease of crystallinity and increase of the acid-solubility in the defective enamel. Those changes could be attributed to the increase of magnesium in the crystal lattice.

**Acknowledgements**

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**References**

9. Trautz OR, klein E, Fessenden E, Addelston HK: The interpretation of the X-ray diffractograms obtained from