Deciduous molar hypomineralisation, its nature and nurture
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MicroCT study on Deciduous Molar Hypomineralisation (DMH)

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Submitted
ABSTRACT

Aim: In this paper, we report the mineral (hydroxyapatite) density of sound and opaque areas in Deciduous Molar Hypomineralisation (DMH) molars with the healthy parts of carious teeth serving as controls.

Materials and methods: Sixteen extracted second primary molars obtained from six children were studied. Five of these molars were DMH molars with yellow opacities, three were DMH molars with white opacities, one was a DMH molar with both yellow and white opacities and seven were molars without DMH. Prior to microCT scanning, the teeth were mounted in impression material (Impregum®) and stored in water with a thymol crystal. Spot analysis and line scans were performed in areas with yellow or white opacities and in sound areas.

Results: The average density of the hydroxyapatite (HA) in the yellow opacities (1245 mg HA/cm$^2$) was significantly lower than in clinically unaffected enamel (1569 mg HA/cm$^2$) of the DMH teeth or of sound molars (1768 mg HA/cm$^2$). The mineral density in the white opacities (1731 mg HA/cm$^2$) was the same as that in the enamel of sound molars (1768 mg HA/cm$^2$). The mineral density values in the yellow enamel opacities (1245 mg HA/cm$^2$) were in between those of dentin (986 mg HA/cm$^2$) and sound enamel (1768 mg HA/cm$^2$).

Conclusion: DMH molars with yellow opacities had a 30% lower mineral density in the hypomineralised enamel compared with unaffected molars. The white opacities do not show a lower mineral content. The total reduction in enamel mineral content in the DMH molars is approximately the same as in white spot lesions, stressing the need for a preventive approach in DMH.
INTRODUCTION

Enamel, the hardest non-vital human tissue, is formed by cells that degrade after the formation of the enamel. Enamel is not remodelled like bone; therefore, disturbances acquired during its development leave a permanent record in the tooth (1). Hypomineralisations of the tooth enamel are observed both in the primary and permanent dentition. Enamel hypomineralisations are defects that occur due to a disturbance during initial calcification and/or during maturation. They are identified visually as alterations in the translucency of the enamel and have a clear border with unaffected enamel. They are variable in severity and can have either a white, yellow or brown colour (2, 3). In the permanent dentition these hypomineralised teeth are known as Molar Incisor Hypomineralisation (MIH) and in the primary dentition they were named Deciduous Molar Hypomineralisation (DMH) (3, 4). In MIH the mineral density of the enamel is reported to determine its mechanical properties (5). Therefore, all hypomineralised parts of the tooth are weaker, and the enamel can chip off easily during regular chewing motions. This weakness may result in post-eruptive enamel loss (2, 3), and caries is also more likely to occur (4). Children with MIH and DMH require more dental treatment and are, as a result, more fearful of dental treatment (6).

No reports are available on the mineral density in DMH molars. In MIH molars, the mineral content has been assessed by microCT. MicroCT, a miniaturised version of the whole body CT scan, is a non-destructive x-ray analysis technique for 3D visualisation at the microscopic level. Analyses can be performed both qualitatively and quantitatively (7, 8). This technique holds promise for measuring mineral densities, which should allow the comparison of the mineral densities of sound enamel, dentine and affected tissues. MIH molars were reported to have a 19-20% reduced mineral density in the affected enamel. In a cross section hypomineralised enamel had a mineral density gradient opposite that of normal enamel, with the lowest mineral density found at the outer surface. Hypomineralised areas were seemed to be distributed randomly throughout the surface of MIH molars, with only the cervical region being less affected. The hypomineralisation defects, however, follow the natural incremental lines of enamel formation. Because no reduction in enamel thickness is found, the defects are not hypoplastic, which suggests a disturbance during the maturation process (5, 7).

As no studies have yet reported on the mineral density patterns of DMH molars, this study aimed to determine mineral density distributions in teeth affected by DMH and in sound control deciduous teeth.
MATERIALS AND METHODS

Participants. Under full anaesthesia, 24 second primary molars were extracted from six children (mean age 5 years, 6 months; 2 girls) referred for dental treatment. All the children had at least one tooth diagnosed with DMH. The teeth were extracted because of pain or for orthodontic reasons. Parents of the children gave permission to further analyse the teeth. Teeth with deep carious lesions or demineralisations of uncertain origin were excluded. One to four teeth per child could be used, in total 16 teeth were included. The selected teeth were mounted together in a block of impression material (Impregum®, 3M ESPE). Each block contained one to four second primary molars from the same child. The teeth were stored in a box containing tap water and thymol crystal to prevent fungal or bacterial growth.

Measures. The teeth were scanned with the µCT 40 (Scanco Medical AG, Brüttisellen, Switzerland). During the scanning procedure, the teeth were kept wet in a cylindrical specimen holder (36.9mm diameter) containing tap water.

The integration time was set at 600 ms, the beam intensity at 70 kV, the current at 114 mA, and the resolution at 0.036 mm. Three-dimensional reconstructions were made with the cone-beam reconstruction algorithm.

After the scanning procedure, which took approximately 1.5 hours per block, the hypomineralised areas were assessed by comparing the scan and the clinical picture. Only areas of the tooth without signs of caries were studied. Measurement points in the enamel and dentin were chosen based on the locations of the yellow or white opacities in the tooth. In areas of sound enamel and dentin, the parts without any sign of caries were chosen (see Figure 5.1). Horizontal and vertical microCT cross sections were studied (Figure 5.1 and 5.2). The measurements were done in one horizontal cross section, which was selected based on the location of the opaque areas. In the case of those teeth with sound enamel, an image of approximately half the crown length was selected. Additional measurements in teeth with yellow opacities were taken coronally and cervically from the opacity. The mineral density found in these locations was within the confidence intervals of the registrations of mineral density left and right of the opacity. Therefore, only the measurements in the horizontal microCT cross sections were used for the results.

Statistics. In all sixteen teeth mineral density of the enamel and dentine were measured at different sites in the teeth (mesial, distal, buccal, lingual). From these measurements means per tooth could be calculated. The mean densities, the median value and the range in the different areas of the tooth were also calculated. The measurements are checked for normal distribution. Statistical analyses were performed with SPSS version 18.0 (SPSS Inc, Chicago, IL, USA) to test differences in mean mineral density with the paired t-test.
Figure 5.1: MicroCT cross section with measurement points (squares and circles) of the horizontal section in the upper third part of the crown in tooth 55.

Figure 5.2: MicroCT cross section of a vertical section in tooth 65. Note the posteruptive enamel loss on the left side (arrow).
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RESULTS

The measured hydroxyapatite densities per tooth are shown in Table 5.1. Differences between the sound areas and opacities were clearly seen. The mean hydroxyapatite density in the yellow opacities (1245 mg HA/cm$^2$) was 21% less than that in the clinically unaffected enamel (1569 mg HA/cm$^2$) in the six DMH molars ($p=0.002$). The density of the enamel in the yellow opacities (1245 mg HA/cm$^2$) was reduced by 30% ($p=0.001$) and the density of the clinically unaffected DMH enamel (1569 mg HA/cm$^2$) was reduced by 11% ($p=0.015$) compared with the mean enamel density of the seven sound molars (1768 mg HA/cm$^2$). In contrast, the mean density of the enamel in the white opacities (1731 mg HA/cm$^2$) was not significantly different from the density of sound enamel (1768 mg HA/cm$^2$) ($p>0.05$).

The density of hydroxyapatite in the yellow opacities was between that of sound enamel and of dentin (sound enamel: 1768 mg HA/cm$^2$; DMH enamel: 1245 mg HA/cm$^2$; sound dentin: 986 mg HA/cm$^2$). In a cross-section of a yellow opacity the outer layer showed a peak in the density (Figure 5.3).

Figure 5.3: Mineral-density measurement of the enamel of tooth 65. Note the peak at the outer enamel surface.
### Table 5.1: Overview of the teeth and hydroxyapatite densities within sound and opaque areas.

<table>
<thead>
<tr>
<th>Child (age) Tooth</th>
<th>Sound Clinically unaffected (min-max)</th>
<th>Clinically unaffected (min-max)</th>
<th>White opacity (min-max)</th>
<th>Yellow opacity (min-max)</th>
<th>Dentin (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (4.1 yrs) 55</td>
<td>1431 (1239-1712)</td>
<td>945 (717-1038)</td>
<td>801 (733-851)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (4.1 yrs) 65</td>
<td>1453 (1387-1526)</td>
<td>1078 (1000-1148)</td>
<td>859 (749-1042)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (4.3 yrs) 55</td>
<td>1642 (1371-1925)</td>
<td>1199 (1098-1364)</td>
<td>963 (930-1004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (4.3 yrs) 65</td>
<td>1539 (1464-1585)</td>
<td>1353 (1329-1376)</td>
<td>919 (767-995)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (4.3 yrs) 75</td>
<td>1788 (1763-2003)</td>
<td>1743 (1659-1912)</td>
<td>1145 (1141-1150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (8.1 yrs) 85</td>
<td>1771 (1526-1989)</td>
<td>1617 (1561-1674)</td>
<td>977 (947-1015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (5.4 yrs) 55</td>
<td>1735 (1610-1847)</td>
<td></td>
<td>1025 (987-1051)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (5.4 yrs) 75</td>
<td>1772 (1694-1911)</td>
<td></td>
<td>985 (940-1033)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (5.4 yrs) 85</td>
<td>1831 (1560-2071)</td>
<td></td>
<td>902 (815-990)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (5.5 yrs) 55</td>
<td>1695 (1540-1889)</td>
<td></td>
<td>1028 (1005-1052)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (5.5 yrs) 65</td>
<td>1717 (1511-2006)</td>
<td></td>
<td>996 (995-996)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (5.5 yrs) 75</td>
<td>1752 (1625-2013)</td>
<td>1892 (1877-1908)</td>
<td>990 (942-1044)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (5.8 yrs) 55</td>
<td>1673 (1522-1857)</td>
<td>1670 (1661-1687)</td>
<td>1040 (938-1141)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (5.8 yrs) 65</td>
<td>1675 (1585-1775)</td>
<td>1463 (1426-1505)</td>
<td>1182 (1115-1258)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (5.8 yrs) 75</td>
<td>1807 (1680-1970)</td>
<td></td>
<td>972 (890-1011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 (5.8 yrs) 85</td>
<td>1816 (1658-1986)</td>
<td></td>
<td>997 (966-1028)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>1768 (53)</td>
<td>1569 (110)</td>
<td>1731 (120)</td>
<td>1245 (207)</td>
<td>986 (94)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>1771 (1695-1831)</td>
<td>1590 (1430-1675)</td>
<td>1643 (1617-1892)</td>
<td>1276 (946-1463)</td>
<td>988 (801-1182)</td>
</tr>
</tbody>
</table>
DISCUSSION

The current microCT analysis revealed that the density of hydroxyapatite in hypomineralised primary molars (DMH) was much lower than in sound enamel. In earlier studies on permanent molars, a reduction of approximately 20% between sound and hypomineralised enamel was found (5, 7). This is quantitatively in line with our findings. Interestingly, the white opacities in DMH molars did not show a reduction in mineral density. This observation agrees with MIH reports, in which the consensus is that darker opacities are weaker (9).

In the permanent dentition, white spot lesions due to caries also have a lower mineral content than sound enamel (74-100% of the mineral content of sound enamel) (10). The mineral content in the hypomineralised molars is therefore comparable with smooth carious white spot areas. The areas with yellow opacities showed the highest reduction in hydroxyapatite density. In this case, the reduction covered the full thickness of the enamel, suggesting that the disturbance causing the hypomineralisation might have extended over a longer period of time (chronic nature) and/or occurred during an early phase of maturation (7, 11).

The higher density in the outer layer of the enamel with yellow opacities has been described previously (5, 7) and could be explained by posteruptive remineralisation of the outer layer or fluoride uptake from the oral cavity surroundings.

Because the number of teeth used in this study is relatively low, the differences in density were considered more important than the absolute density values. Previous reports on MIH molars also included a limited number of teeth, similar to this study, but showed clear changes in the density of the enamel (5, 7, 9). We note that it is relatively difficult to get access to affected primary teeth, also given their prevalence.

The age of the children at which the teeth were lost likely also influenced the enamel density as fluoride could have been incorporated in the outer layer of enamel for several years (12).

This report is the first to describe the density of the hypomineralisations in DMH molars. The enamel in the second primary molars is now confirmed to be hypomineralised, justifying the clinically assessed name of DMH. Studies on MIH molars, such as that performed by Fearne et al. (7), showed a very comparable picture of hypomineralisation. DMH was also clinically resembled in the mineral content of the enamel of MIH molars.

The clinical consequences of the lower mineral content can obviously lead to a more rapid onset of caries. However, the lower mineral content can also cause a higher sensitivity to temperature changes. The DMH molar can cause pain, even in the absence of caries. Because the DMH molars have approximately the same reduced mineral content as white spot lesions, the clinical treatment of DMH can reasonably be assumed to be in line with the treatment of white spot lesions.
CONCLUSION

DMH molars with yellow opacities had a 30% lower mineral density in the hypomineralised enamel compared with unaffected molars. The white opacities did not show differences between clinically unaffected and hypomineralised areas in the DMH molar. The total reduction in enamel mineral content in DMH molars is approximately the same as in white spot lesions, stressing the need for a preventive approach in DMH. Our observations confirmed that enamel in the second primary DMH molars did indeed have a lower mineral content, justifying the clinically assessed name of DMH.
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LITERATURE


