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MicroCT study on the enamel mineral density of primary molars

ABSTRACT

Aim *The aim of this study is to report on the mineral density of the enamel of primary molars related to the age of the child and to compare the mineral density of sound and carious enamel in those molars.*
Materials and methods *This study included 23 children and 41 extracted primary molars. The primary molars of 21 children met all of the inclusion criteria, and these were studied and scanned using microCT. The teeth were embedded in Impregum® (3M ESPE) and stored in a solution of tap water with thymol crystals. Sixteen primary molars from 7 children were used to compare the mineral density in sound and carious areas, and 13 primary molars from 11 children were used for the comparison between mineral density and time in situ.*
Results *A statistically significant difference (31%) was found between the mineral density in carious enamel and sound enamel (p = 0.0006). In addition, a significant relationship was observed between the mineral density of sound enamel and the time the teeth had been in situ (r = 0.698). We also found two teeth with radiolucencies in the dentin with the enamel clinically showing only a non-cavitated carious lesion in the enamel. No significant differences were found between the mean mineral density in sound enamel surfaces and unaffected areas in surfaces of molars with enamel caries (p = 0.4373).*
Conclusion *Local and general differences in enamel mineralisation are presented. Post-eruptive maturation seems to be present not only in permanent teeth but also in primary molars. Carious enamel has significantly less mineral density than clinically sound enamel.*

Keywords *Caries; Enamel; microCT; Primary dentition.*

Introduction

After completion of mineralisation and eruption into the oral cavity, the outer surfaces of teeth undergo changes, especially because they are exposed to the oral cavity and will be affected by local conditions and dietary habits [ten Cate, 1998]. This outer surface is exposed to a dynamic process of demineralisation and remineralisation depending on the critical pH value and the presence of calcium, phosphate and fluoride [Ten Cate, 1990; Ehrlich et al., 2009; Van Loveren, 2007]. In addition, the hardness of enamel increases with the time of exposure to the oral cavity, indicating a process of 'post-eruptive maturation'. The first post-eruptive years are the most important because of the less favourable mineral content and low ability to perform optimal oral hygiene [Elfrink et al., 2006; Cardoso et al., 2009].

Compared to permanent teeth, primary teeth are more susceptible to demineralisation and dentin caries. This is due to a different anatomy; a thinner layer of enamel [ten Cate, 1998], larger proximal contact surfaces and lower micro hardness due to a lower degree of mineralisation, a higher water content and higher permeability [Taji and Seow, 2010]. These characteristics, and the fact that children have less saliva flow and a low calcium concentration in their saliva compared to adults [Anderson et al., 2001] make primary teeth more prone to the initialisation and progression of caries. Mineral density can be assessed by microCT scans, which enable measurement of sound and affected dentin and enamel. Recently, Soviero et al. [Soviero et al., 2012] concluded that microCT can be used as a gold standard for in vitro detection of proximal carious lesions in primary molars [Soviero et al., 2012]. They also concluded that the assessment of the depth of caries in enamel and dentin is highly accurate when microCT is used.

The degree of mineralisation and chemical content vary between individual teeth and influence the demineralisation depth [Sabel et al., 2012]. It is also possible that some molars are more caries-prone because of insufficient mineral density due to developmental defects such as Hypomineralised Second Primary Molars (HSPM) [Elfrink et al., 2006; Elfrink et al., 2010; Elfrink et al.,

2013; Ghanim et al., 2013]. Variations in mineral density may well increase the sensitivity to demineralisation and caries. Therefore, the aim of this study is to report on the mineral density in both sound and carious areas of enamel in primary molars and to find support for post-eruptive changes in mineralisation in primary teeth.

Materials and methods

For this study, 41 primary molars were used. These teeth were extracted from 23 children. The treatment took place under general anaesthesia due to the (mostly age-related) limited co-operation of the children. Extractions were indicated because of caries, hypomineralisation or orthodontic reasons. Parents were informed and gave permission for using the extracted primary molars in this study. Six teeth were excluded before scanning because of Hypomineralised Second Primary Molars (HSPM) [Elfrink et al., 2008].

The distribution of age, mineral content and months *in situ* of the teeth was checked for the 21 children in whom at least one molar was selected to be scanned.

The included teeth were mounted with their roots in a block of impression material (Impregum® 3M ESPE). Each block included the primary molars of one child. The blocks were stored in a box containing tap water and thymol crystals to prevent fungal and bacterial growth.

The teeth were scanned with a µCT 40 (Scanco Medical AG, Brüttisellen, Switzerland). This scanner is calibrated weekly using a phantom with densities ranging from 0 to 800 mg HA/cm^3 [van Ruijven et al., 2007]. During the scanning procedure, the blocks with the teeth were held in plastic tubes (36.9 mm diameter) containing tap water, and the top piece was occluded with Scotch bond.

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, carious and sound areas were assessed by comparing the scan and the clinical picture. Thirteen molars with only cavitated caries lesions extending to the dentin were excluded.

Children with primary molars with at least one surface with a radiolucency restricted to the enamel and another tooth with a sound enamel surface were included in the test group. This way, only seven children with 16 molars were included in this part of the study. Measurement points of carious enamel and sound dentin were selected by using radiolucencies restricted to the enamel that were not cavitated. The slice in which the enamel radiolucency was at its largest size was selected for measurement. In figure 1, the microCT slide and the clinical view of the opacity in the enamel are shown.

The slide for measurements in the sound enamel surface was chosen in an area of the tooth close to the slide of the teeth with radiolucencies. This was performed to obtain a good comparison between the two. The measurement points were established on horizontal microCT cross-sections. The mean mineral density was calculated for every measurement point.

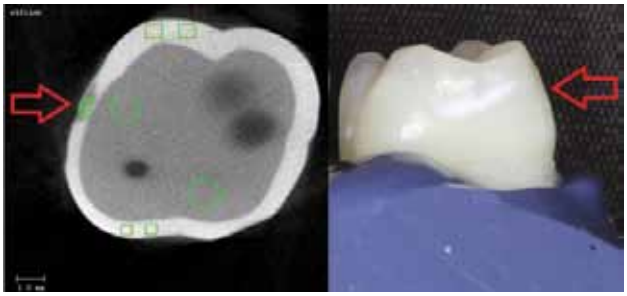

FIG. 1 Left: MicroCT cross section with measurement points of tooth 55 with radiolucency in the enamel on the mesial surface (red arrow). Right: Photograph of the same tooth embedded in Impregum ® (3M ESPE) with white opacity in the enamel (red arrow).

Teeth	Range	Mean age
54/64	13-19 months	16 months
55/65	25-33 months	29 months
74/84	14-18 months	16 months
75/85	23-31 months	27 months

TABLE 1 Mean age of the child and its range (in months) of the eruption of primary molars (JADA, 2005).

To investigate the relationship between months *in situ* and mineral content, 13 primary molars with sound enamel surfaces from 11 children were included. It is difficult to report the exact period of months *in situ* for each tooth; neither the parents, the child nor the dentist knows the exact eruption time of an individual tooth. Because of the wide range in eruption time of the primary teeth in children, we calculated the mean age as described in the Journal of the American Dental Association in 2005; 16 months for the first molars, 27 months for lower second molars and 29 months for upper second molars (Table 1). We calculated the time *in situ* by subtracting the mean eruption time from the age (at the moment of extraction).

Statistical analyses

A paired T-test was used to see if there were significant differences in the mineral content of the control and test groups ($\alpha = 0.05$).

Pearson correlation coefficients were calculated for the relationship between mineral density in sound enamel and months *in situ* of the primary molars ($\alpha = 0.01$).

Statistical analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

The study included 23 children (ages: 3 years 5 months

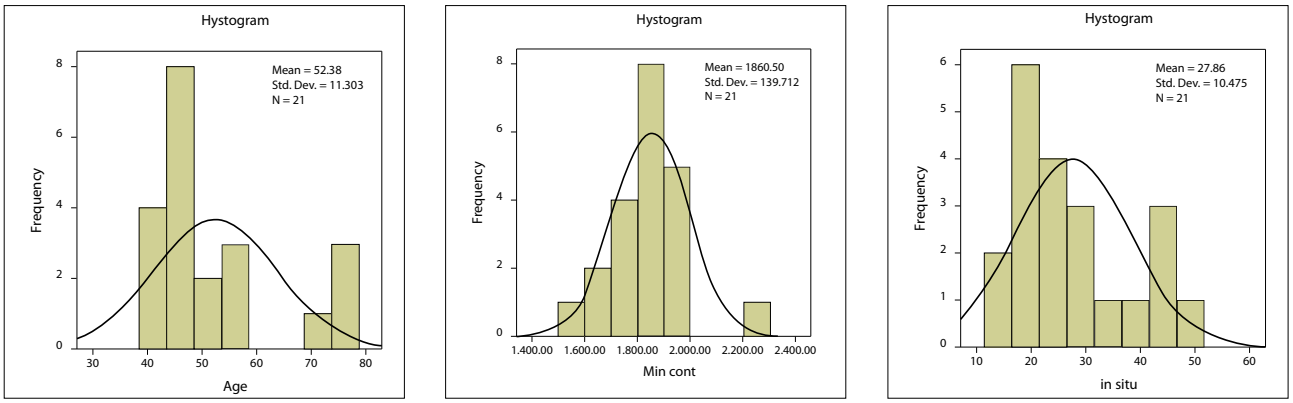

FIG. 2 Distribution of mean ages in months (a), mineral content in mg HA/cm^3 (b) and time *in situ* in months (c). Note the normal distribution of these variables.

Child number	Age	Tooth	Control	In situ	Sound enamel controls min/max	Sound enamel min-max	Radiolucent enamel min-max	Sound dentin min-max
2		75	Y		1793			1032
	47			20	1662-1941			1010-1083
2		85	N			1898	1138	988
	47			20		1833-1953	1129-1142	938-1058
6		55	Y		1954			1209
	55			26	1884-1995			1177-1260
6		85	N			1986	1547	1178
	55			28		1961-2026	1483-1628	1193-1149
10		64	Y		1963			1126
	45			29	1906-2029			1076-1179
10		54	N			1856	1236	1117
	45			29		1769-1940	1124-1348	1096-1146
13		54	Y		1840			1068
	42			26	1785-1887			1055-1086
13		64	N			1944	1300	1103
	42			26		1884-2016	1187-1414	1078-1127
18		85	Y		1763			1095
	41			14	1624-1937			1060-1121
18		75	N			1677	1227	970
	41			14		1580-1991	1200-1251	915-1035
19		85	Y		1710			1029
	48			21	1482-1922			1001-1087
19		65	N			1856	1574	1078
	48			19		1803-1963	1559-1590	1078-1079
23		75	Y		2208			1340
	74			47	2191-2219			1318-1368
23		55	N			1838	1209	1094
	74			45		1761-1902	1284-1133	1060-1168
23		65	N			1950	1164	1013
	74			45		1901-1988	1137-1224	996-1030
MEAN				30	1930	1876	1299	1096
STANDARD DEVIATION				12	192	96	169	94
MEDIAN				28	1897	1877	1232	1094
P				ref		0.4373	0.0006*	-

TABLE 2 Ages (in months) and period *in situ* (see also table 1), mineral density in mg HA/cm^3.

- 6 years 2 months) and 41 primary molars. Thirteen molars with dentin caries only and six molars with HSPM were excluded from all analyses. The measurements of the ages, mineral content and months *in situ* had normal distributions (Fig. 2).

Most primary molars had enamel lesions on their

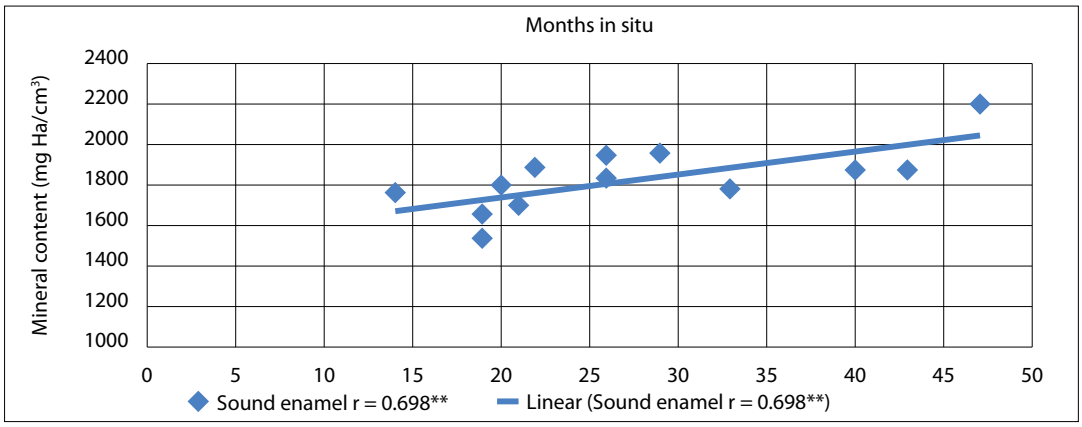

FIG. 3 Graph showing the relationship and the correlation coefficients between mean mineral content and months *in situ*.

mesial surfaces except for one, which had an enamel lesion on the lingual surface. Children with at least one molar with radiolucent enamel and another molar with a sound enamel surface are shown in table 2. As mentioned above, only 7 children with 16 primary molars were included in this test group.

The results from the paired T-test (Table 2) showed no significant differences between the sound enamel of the control and test groups ($p = 0.4373$). The mean mineral content was 3% lower in the test group than in the control group (1876 mg HA/cm^3 versus 1930 mg HA/cm^3), but this was not significant. The mean mineral content of the radiolucent areas in the enamel was significantly lower ($p = 0.0006$), which means that carious lesions in the enamel contained 31% less mineral than the sound enamel of the same tooth.

Figure 3 shows the relationship between enamel mineral content and the number of months *in situ*. The correlation coefficient was 0.698 ($p = 0.008$) for the sound enamel surfaces of the 13 primary molars, which was significant at $\alpha = 0.01$.

After observing this significant relationship between months *in situ* and enamel mineral content, we corrected the analysis of the differences in mineral content for 'months *in situ*'. The difference between the mineral contents of the sound enamel of the control and test groups remained insignificant (data not shown).

Anecdotic in this study, two molars were noted as having a radiolucency in enamel and dentin, but were without clinical signs of a cavity, as shown in figures 4 and 5. The photographs were taken after the microCT scanning procedure. The surfaces with dentin and enamel radiolucencies were studied with light and probe. Only white, yellow or brown opacities were observed in these surfaces, with no irregularities in the enamel.

Discussion

This study shows that the mineral density in carious enamel is a site-specific process, resulting in a mineral reduction of 31% compared to sound enamel surfaces in the same tooth. Additionally, a generalised relationship between mineral density and months *in situ* has been found. Correction for months *in situ* did not change the results of the other finding.

Studies on mineral density in primary molars are scarce. In the permanent dentition, white spot lesions due to dental caries have an up to 26% lower mineral content than sound enamel [Cochrane et al., 2012]. A study by Huang et al. showed an up to 40% lower mineral content in lesions in the enamel of premolars [Huang et al., 2007]. The 31% that we found in this study is in the same range. In addition, post-eruptive maturation plays a significant role in this outcome. In this study, we found a significant

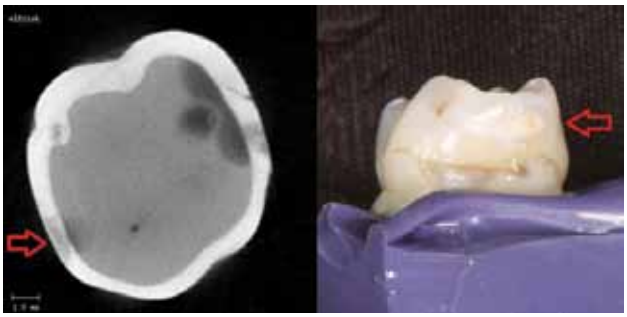

FIG. 4 Left: MicroCT cross sections of tooth 55. Note the radiolucency in the enamel and dentin with no structure loss of enamel (red arrow). Right: Photograph of the same surface denoted by the red arrow. Only white/yellow/brown opacity is observed, with no irregularities in the enamel.

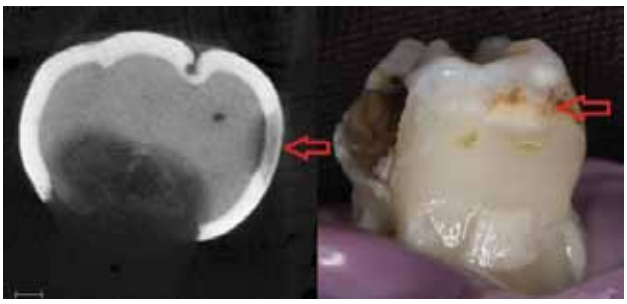

FIG. 5 Left: MicroCT cross section of tooth 75. Again, note the radiolucency in the enamel and dentin without any structure loss of enamel (red arrow) not connected with the large lingual cavity. Right: Photograph of the same surface denoted by the red arrow. Only white/yellow/brown opacity is observed, with no irregularities in the enamel.

correlation coefficient of 0.698 between the mineral density of sound enamel and the number of months *in situ*. Earlier studies have shown a significant difference in enamel hardness between unerupted teeth and teeth exposed for more than 10 years in the permanent dentition [Palti et al., 2008]. Furthermore, significant differences were found at different post-eruptive ages, which might be related to the incorporation of calcium, phosphate and fluoride from the oral environment [Cardoso et al., 2009]. One study showed shallower lesions in individuals more than 8.2 years old, which was interpreted as a post-eruptive maturation of enamel [Sabel et al., 2012]. In another study in primary molars that measured enamel mineral content by X-ray microtomography, a higher mineral concentration was found along the exposed surface compared to that at the amelodentinal junction of the same tooth [Wong et al., 2004]. These studies support our findings regarding post-eruptive maturation in primary dentition. We also found two teeth with radiolucencies in enamel and dentin without clinically visible cavities. The carious lesions could have progressed far into the dentin without any clinical signs, as observed in this study. The lesion depth is also influenced by the chemical composition of the enamel [Sabel et al., 2012]. A deeper lesion will be found if the porosity of the enamel is higher [Sabel et al., 2012]. However, a deeper lesion does not always cause enamel breakdown, so the lesion can progress into dentin without the clinical finding of a cavity. If no enamel breakdown occurs, demineralisation might be masking the caries process, but this is a subject for further studies.

One recent study concluded that measurements from microCT can be used as a gold standard for detecting proximal caries lesions in primary molars [Soviero et al., 2012]. However, the microCT can only be used for extracted molars. Thus, in this study we were limited to molars that needed to be extracted from young children. Therefore, most molars had deep cavities, and only some sound molars were extracted by the paediatric dentists due to space management. The number of molars that can be used in this research is limited because in general, restoration is preferred to extraction, and at least one sound surface of a tooth with enamel caries needed to be present. Inclusion of teeth without dentin caries and only enamel caries and sound teeth would have been preferred, but this is difficult to realise because these teeth are rarely extracted. In fact, only for orthodontic treatments are sound or nearly sound molars extracted. These children are in general a bit older than the children in our study. The mineral density in areas of enamel in teeth with or without enamel caries was not significantly different in this study. In children with several extractions and a large difference in the severity of the caries lesions in the extracted teeth, the mineral content of these teeth was found to be nearly the same. Nevertheless, the results can include bias. However, this means that the mineral density can play an important role in the caries process. In further research, the mineral density in primary molars without caries or with only enamel caries could be studied. More studies on the enamel density of primary molars are needed. The process of post-eruptive maturation of the enamel in primary molars also needs confirmation in further studies.

Conclusion

This study shows a significant difference between clinically sound and carious enamel and creates evidence for the mechanism of post-eruptive maturation in primary molars. The enamel mineral density does not differ between areas in teeth with or without enamel caries. Intriguing observations were made on proximal caries in dentin that could occur without enamel cavitation.

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