

## A Study of Primary Dental Enamel From Preterm and Full-term Children Using Light and Scanning Electron Microscopy

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### Abstract

**Purpose:** The aim of this study was to examine the enamel thickness of the maxillary primary incisors of preterm children with very low birth weight (<1,500 g) compared to full-term children with normal birth weight.

**Methods:** A total of 90 exfoliated maxillary primary central incisors were investigated using light microscopy and scanning electron microscopy (SEM). Three serial buccolingual ground sections of each tooth were examined under light microscopy, and maximum dimensions of the prenatally and postnatally formed enamel were measured.

**Results:** The enamel of preterm teeth was approximately 20% thinner than that for full-term teeth. Most of the reduction was observed in the prenatally formed enamel. This was 5 to 13 times thinner than that for full-term children ( $P < .001$ ). The "catch-up" thickness of postnatally formed enamel did not compensate fully for the decrease in prenatal enamel ( $P < .001$ ). Although none of the teeth used in this study had enamel defects visible to the naked eye, 52% of preterm teeth showed enamel hypoplasia under SEM, compared with only 16% found on full-term teeth ( $P < .001$ ). These defects were present as pits or irregular, shallow areas of missing enamel.

**Conclusions:** Preterm primary dental enamel is abnormal in surface quality, and is significantly thinner compared to full-term enamel. The thinner enamel is due mainly to reduced prenatal growth and results in smaller dimensions of the primary dentition. (Pediat Dent 2005;27:374-379)

**KEYWORDS:** DENTAL ENAMEL, PRETERM CHILDREN,  
PRIMARY TEETH, NEONATAL LINE, ENAMEL THICKNESS

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It is now well known that prematurely born children experience many oral complications associated with their preterm births.<sup>1-6</sup> Enamel hypoplasia has been reported to be present in over 70% of preterm children. This condition is likely the result of:

1. neonatal derangements in mineralization due to poor supply and absorption of calcium and phosphate;
2. local trauma associated with laryngoscopy and endotracheal intubation.<sup>1-7</sup>

In addition, short-term distortions of the palate may be caused by prolonged endotracheal intubation, which is frequently required for pulmonary ventilation of the preterm

children.<sup>5-7</sup> As in other aspects of growth, preterm children also show delayed dental development and eruption in early childhood, but compensatory catch-up growth occurs in later childhood.<sup>2</sup>

Preterm children have reduced dental dimensions, compared to full-term children. In a controlled study using natural primary teeth, Seow and Wan<sup>8</sup> showed that natural primary incisors from preterm children are significantly smaller in both mesiodistal and faciolingual dimensions compared to those from full-term children. From plaster casts of the primary dentition, Fearne and Brook<sup>9</sup> reported smaller sizes of the primary molars. The reduced dimensions are presumably the result of serious derangements of metabolic functions associated with their preterm births. It is unclear, however, whether the size reduction is due to a generalized reduction in crown dimension, or whether it is the result of a reduction in enamel thickness.

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**Table 1. Demography of Subjects Who Donated Teeth for Study**

	Preterm (N=45)	Full-term (N=45)	P value
Boys (%)	25 (56%)	25 (56%)	NS*
Girls (%)	20 (44%)	20 (44%)	NS*
Total (100%)	45 (100%)	45 (100%)	NS*
Birth weight (g±SD)	1,480±320 g	3,370±300 g	<.01†
Gestational age (wks±SD)	32.3±3.5	39.2±2.1±0.32 wks	<.01†
Right-side incisors 50 (100%)	23 (51%)	27 (60%)	NS*
Left-side incisors 40 (100%)	22 (49%)	18 (40%)	NS*

\*Chi-square test.

†Student's *t* test.

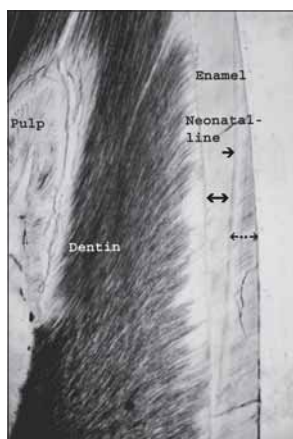


Figure 1. Photograph of section of a preterm tooth showing the neonatal line (single-arrowhead), prenatal enamel (solid line), and postnatal enamel (dotted line).

The authors hypothesize that a reduction of enamel thickness in the teeth of preterm children contributes to their smaller dental dimensions. Thus, the present study aimed to compare the thickness of primary enamel in preterm and full-term children by examining the relative amount of enamel formed before and after birth in histological sections of primary central incisors.

To further support the authors' hypothesis that enamel is abnormal in the preterm children, scanning electron microscopy (SEM) studies were also carried out on other incisors of preterm

and full-term control children to detect differences in enamel surface quality.

## Methods

This study was approved by the Human Ethics Review Committee, University of Queensland, Brisbane, Australia. Naturally exfoliated primary teeth were donated from parents of preterm children who were cohorts undergoing multidisciplinary assessment at the Growth and Development Clinic, Mater Children's Hospital, South Brisbane, Australia. Full-term (control) children of normal birth weight attending the Pediatric Dentistry Clinic of the School of Dentistry, University of Queensland, also donated exfoliated teeth for this study. As shown in Table 1, the mean birth weights and gestational ages of the preterm vs full-term children were 1480±320 g vs 3370±320 g ( $P<.01$ ), and 32.3±3.5 weeks vs 39.2±2.1±0.32 weeks ( $P<.01$ ), respectively.

In order that comparisons could be made directly between groups, only maxillary primary central incisors were used in this study. One tooth was donated from each child in the study. Furthermore, to eliminate the effects of secondary changes on enamel, such as dental caries or

traumatic fracture, only those teeth without obvious enamel changes when examined with the naked eye were used for study. For the light microscopy study, 40 primary maxillary incisor teeth (20 each from preterm and full-term children) were selected. For the SEM study, another 50 teeth (25 each from preterm children and full-term children) were selected. There were approximately equal numbers of teeth from boys and girls in the preterm and full-term groups, respectively. The

numbers of right- and left-sided teeth were also equal in numbers (Table 1).

## Light microscopy

The teeth were cleaned with a bristle brush and pumice and stored dry. For light microscopy, the teeth were sectioned faciolingually in the mid-sagittal plane using a hard tissue saw microtome. Three serial ground sections, each 80-µm thick, were prepared from each tooth.

The sections were viewed under a light microscope (Olympus, Tokyo, Japan) at ×200 magnification. In each section, the neonatal line in the enamel was located as a line of visible change of direction of the enamel rods in the enamel, delineating the enamel formed before and after birth (Figure 1). The distance from the cemento-enamel junction (CEJ) at the buccal aspect to the commencement of the neonatal line was measured using an eyepiece which contained a measuring grid.

For each section, the widths of prenatally and postnatally formed enamel, respectively, were measured at right angles to the dentinoenamel junction (DEJ) at 2 points from the CEJ: (1) 2.50 mm; and (2) 1.25 mm. The maximum thickness of enamel at the facial aspect was also measured. For each tooth section, the measurements were taken in duplicate, and a mean and standard deviation were obtained for each tooth.

## Scanning electron microscopy

For the SEM studies, the teeth were:

1. cleaned in an ultrasonic bath containing 0.5% sodium hypochlorite solution for 30 minutes;
2. dried with compressed air;
3. vacuum coated with silver.

The SEM was performed using a Philips SEM 505 (Philips Electronics, Eindhoven, Netherlands) scanning electron microscope kept at the Centre for Electron Microscopy at the University of Queensland. The teeth were scanned at both low and high power. To standardize images from preterm and control groups, the SEM photomicrographs were compared at ×20 and ×200 magnifications.

## Statistical analysis

The student's *t* test was used for statistical analysis. The alpha value was set at 0.05.

**Table 2. Comparison of Neonatal Line in Preterm and Full-term Primary Maxillary Central Incisors**

	Primary central incisor		Difference	P value*
	Preterm N=20 (10 males, 10 females)	Full-term N=20 (10 males, 10 females)		
Distance from CEJ† at which neonatal line commences; mean mm±SD	0.223±1.27	1.41±0.61	0.82±0.66	<.01
Length of neonatal line; mean mm±SD	2.60±1.16	2.92±3.81	0.32±2.65	>.1 (NS)

\*Student's *t* test.

†Cemento-enamel junction.

### Results

Table 2 compares the lengths and locations of the neonatal line in preterm and full-term teeth as seen under light microscopy. As shown in Table 2, the length of the neonatal line was not significantly different between preterm and full-term teeth (2.60±1.16 mm vs 2.92±3.81 mm; *P*>.1), suggesting that the length of the preterm tooth crowns is not significantly different from that of full-term teeth. The distance from the CEJ at which the neonatal line commences, however, is increased in the full-term by 0.82 mm (*P*<.01).

Table 3 compares the thickness of the pre- and postnatally formed enamel in preterm and full-term teeth under light microscopy. As shown in Table 3, the mean maximum facial thickness of enamel from the preterm incisors was significantly less than that for the full-term children (0.423±0.057 mm vs 0.505±0.091 mm, full-term:preterm ratio=4.71; *P*<.001). At 2.500 mm from the CEJ, the mean thickness of prenatally formed enamel was only 0.016±0.043 mm in the preterm children compared to 0.219±0.101 mm in full-term children. The full-term:preterm ratio was 13.45 (*P*<.001). The postnatally formed enamel, however, was twice as thick in the preterm children (0.318±0.066 mm) compared to the enamel of full-

term children (0.166±0.118 mm; full-term:preterm ratio=0.52; *P*<.001).

Despite this increase in postnatal enamel thickness, the greatest enamel thickness of preterm enamel was still less than that for full-term children at this distance from the CEJ (0.334±0.066 mm vs 0.385±0.118 mm; full-term:preterm ratio=1.15; *P*<.01). At a distance of 0.1250 mm from the CEJ, the prenatal enamel in full-

term children was nearly 5 times thicker than that for the preterm children (0.043±0.037 mm vs 0.009±0.004 mm; *P*<.001). At this short distance from the CEJ, however, although the preterm enamel also showed thicker postnatal enamel, there were no significant differences between preterm and full-term children (0.248±0.041 mm vs 0.234±0.074 mm).

Under SEM, of the 25 preterm central incisors scanned, 13 (52%) showed evidence of surface enamel hypoplasia, compared to only 4 out of 20 teeth (16%) in the full-term group (*P*<.01; Table 4). As shown in Figure 2, the defects were mainly located in the mid-crown and incisal third regions of the crowns. The majority of defects presented as patchy, shallow areas of surface enamel loss. Within these defects, the lesions showed small pits. Higher magnification showed defective enamel with irregularities of prism formation. In other areas, the hypoplastic enamel showed no evidence of prism formation and the enamel surface appeared granular (Figure 2). By contrast, enamel from full-term children generally showed unremarkable surfaces, except for a few scratches (Figure 3). Normal enamel from the full-term teeth usually showed prism-free (aprismatic) enamel.<sup>11,12</sup>

**Table 3. Comparison of the Thickness of Pre- and Postnatally Formed Enamel in the Maxillary Primary Incisors of Preterm and Full-term Children**

	Prenatally formed enamel thickness (mean mm±SD)			Postnatally formed enamel thickness (mean mm±SD)			Total enamel thickness (mean mm±SD)		
	Preterm	Full-term	Full-term: Preterm ratio	Preterm	Full-term	Full-term: Preterm ratio	Preterm	Full-term	Full-term: Preterm ratio
Measured at 0.125 mm from the CEJ†	0.009 ±0.004	0.043 ±0.037	4.71 ( <i>P</i> <.001*)	0.248 ±0.041	0.234 ±0.074	0.94 ( <i>P</i> >.1; NS)	0.257 ±0.041	0.277 ±0.074	1.078 ( <i>P</i> <.001)
Measured at 0.250 mm from the CEJ†	0.016 ±0.043	0.219 ±0.101	13.45 ( <i>P</i> <.001*)	0.317 ±0.066	0.165 ±0.118	0.52 ( <i>P</i> <.001)	0.334 ±0.066	0.385 ±0.118	1.154 ( <i>P</i> <.001)
Maximum thickness of enamel at facial aspect							0.422 ±0.057	0.505 ±0.091	1.195 ( <i>P</i> <.001)

\*Student's *t* test.

†Cemento-enamel junction.

**Table 4. Distribution of Enamel Hypoplasia in the Primary Maxillary Incisors of Preterm and Full-term Children**

Location of enamel hypoplasia	Preterm (N=25)	Full-term (N=25)	P value
Mainly cervical one third	1 (4%)	2 (50%)	
Mainly middle and incisal	8 (32%)	2 (50%)	
Mainly incisal	1 (4%)	0	
Entire crown affected	3 (12%)		
Total with enamel hypoplasia	13 (52%)	4 (16%)	<.001
Total without enamel hypoplasia	12 (48%)	21 (84%)	<.001

## Discussion

The present study's results confirmed the hypothesis that, compared to full-term children, preterm children have decreased enamel thickness of the primary maxillary incisors. Prenatally formed enamel may be delineated from the postnatally formed enamel by the presence of the neonatal line—a histological landmark consisting of altered arrangement of the enamel prisms which results from the transition of intrauterine to extrauterine environments.<sup>13</sup> In the preterm children, the prenatally formed enamel is the most reduced—at a level of approximately 5 to 13 times the thickness of the enamel of full-term children. This directly reflects the shortened duration in the prenatal stage of enamel formation. Interestingly, the discrepancy is partially compensated by an increase in postnatal enamel formation of between 6% and 90% percent. The overall thickness of the facial enamel of the maxillary central incisors, however, is still significantly less than that for full-term children by approximately 20%.

It is now well known that the size of dental crowns is thought to be determined by both genetic and environmental factors, but the relative importance of these influences is unclear. In previous studies, the authors reported that pre-

maturity of birth is associated with smaller primary teeth 6% to 11% of the time, compared to the teeth of full-term children.<sup>8</sup> This crown size reduction may be the result of a generalized reduction in tooth size and may reflect an overall smallness in body size resulting from general growth retardation associated with birth prematurity.<sup>18-20</sup> Thus, growth retardation may occur as a result of the severe metabolic derangements encountered by a preterm child and is likely to be reflected in reduced and abnormal mitotic activity of all the tissues mapping out the amelodentinal junction in 3 di-

mensions of the dental crown. Such a situation is observed in many adverse maternal and fetal conditions, such as maternal smoking, hemifacial microsomia, congenital endocrine abnormalities (eg, thyroxine and growth hormone deficiencies), and chromosomal abnormalities in which smaller crown dimensions have been reported in both the primary and permanent dentition.<sup>14-17,21</sup>

By contrast, the authors hypothesize that observed reductions of primary crown size of preterm children results mainly from reduced enamel formation, most likely the result of premature cessation of ameloblast activity induced by the severe systemic derangements at birth. The site of this cessation of activity is marked by the neonatal line, which was found to be located further incisally from the CEJ in preterm teeth compared to full-term teeth. This finding affirms the neonatal line as a suitable reference location to delineate prenatally and postnatally formed enamel.<sup>10,13</sup>

The authors' hypothesis is strongly supported by the present study's results, which show a significant reduction in overall enamel thickness within the same order of dimension reduction of the maxillary primary incisors of preterm children in previous studies.<sup>8</sup> It is of interest to note

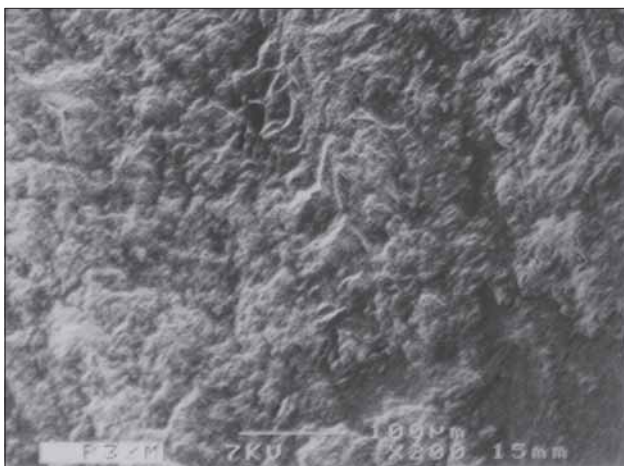


Figure 2. Scanning electron microscope image of preterm enamel showing surface enamel hypoplasia. The enamel appears rough, granular, and poorly mineralized (magnification  $\times 200$ ). Bar represents 100  $\mu\text{m}$ .

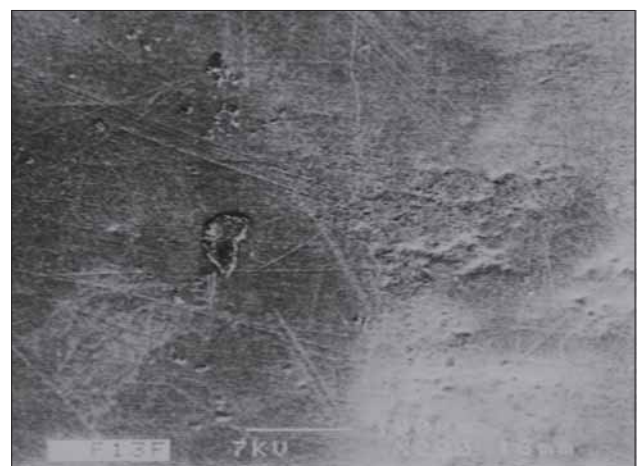


Figure 3. Scanning electron microscope image of enamel surface of incisor from full-term child. The surface is unremarkable, except for a few scratches (magnification  $\times 200$ ). Bar represents 100  $\mu\text{m}$ .

that, while there is increased growth of postnatal enamel to compensate for the severely reduced prenatal enamel development, recovery of enamel formation is not sufficient to achieve full thickness enamel formation in the present study cohort of preterm children who had mean birth weights of <1,500 g and below.

In addition, the derangement of the ameloblasts caused by the severe metabolic insults is likely to be worsened by the reduced supply of calcium and phosphate to the developing teeth. It is well known that preterm children have severely impaired calcium and phosphate metabolism, often to the extent that many extremely preterm neonates show signs of clinical rickets.<sup>22,23</sup> The deprivation of mineral is likely to be worse in the case of the forming teeth, as maintenance of constant blood chemistry and bone mineralization probably take precedence over the dentition for supply of calcium and phosphate.<sup>24</sup>

Although it is current standard medical practice to increase the supply of minerals to preterm children through the use of milk fortification and other techniques, the problem is difficult to manage. This is due to the prematurity of the preterm infant's metabolic systems for absorbing and processing minerals.<sup>22,23</sup> In previous publications, the authors showed that children with premature neonatal rickets are highly predisposed to enamel hypoplasia and that there was a strong association of osteopenia of prematurity with abnormal enamel mineralization.<sup>3,24</sup> Thus, the authors hypothesize that the reduced enamel in preterm children is likely to have resulted from both the cessation/reduction of ameloblastic activity and the reduced supply of mineral to the developing teeth. These effects are more likely to be associated with prematurity than low birth weight.

The present study also shows that 52% of preterm incisors with no visibly discernable defects demonstrated surface enamel hypoplasia detectable at the SEM level. These findings suggest that enamel quality is affected, despite the general compensatory growth of the tissue during the postnatal period. The location of the majority of defects at the middle and incisor regions of the teeth further suggests that the time period of the insult to enamel matches the events of the premature births.

Because dentin dimensions were not measured in the present study, a reduction in overall dental size cannot be entirely excluded. It is reasonable to assume, however, that genetic influences on dental development are not associated with birth prematurity. The genetic parameters of crown shape and other dental tissues are not likely to be affected by the preterm birth. Furthermore, in the present study, there are equal numbers of teeth from males and females in the preterm and full-term groups, so that sex differences in enamel formation are also excluded.

Significant clinical implications arise for the thinner and hypoplastic enamel found in preterm children. In previous studies, the authors reported increased caries susceptibility of preterm children and demonstrated the strong association between enamel hypoplasia and dental caries.<sup>25,26</sup> The in-

creased caries among children with enamel hypoplasia is likely a result of greater attachment of mutans streptococci to rough surfaces<sup>27</sup> and promoting earlier colonization of the cariogenic bacteria.<sup>28</sup>

## Conclusions

Based on this study's results, the following conclusions can be made:

1. Preterm primary dental enamel is abnormal in surface quality and is significantly thinner than full-term enamel.
2. The thinner preterm enamel is due mainly to reduced prenatal growth.
3. The reduced enamel is likely to result in smaller crown dimensions in preterm children.

## Acknowledgements

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