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Polarized light and scanning electron microscopic investigation of enamel hypoplasia in primary teeth

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Background. Enamel hypoplasia is a developmental disturbance during enamel formation, defined as a macroscopic defect in the enamel, with a reduction of the enamel thickness with rounded, smooth borders. Information on the microstructural level is still limited, therefore further studies are of importance to better understand the mechanisms behind enamel hypoplasia.

Aim. To study enamel hypoplasia in primary teeth by means of polarized light microscopy and scanning electron microscopy.

Methods. Nineteen primary teeth with enamel hypoplasia were examined in a polarized light microscope and in a scanning electron microscope.

Results. The cervical and incisal borders of the enamel hypoplasia had a rounded appearance, as the prisms in the rounded cervical area of the hypoplasia were bent. The rounded borders had a normal surface structure whereas the base of the defects appeared rough and porous.

Conclusions. Morphological findings in this study indicate that the aetiological factor has a short duration and affects only certain ameloblasts. The bottom of the enamel hypoplasia is porous and constitutes possible pathways for bacteria into the dentin.

Introduction

There are a wide variety of terms and definitions used to describe different enamel defects[1]. Of special interest is the external enamel hypoplasia, often in the literature called linear enamel hypoplasia. This may be defined as a macroscopic defect of the enamel, involving the surface with a reduced enamel thickness and rounded, smooth borders; the base of the defect often being rough[2–4]. The macro and microscopical appearances suggest that only some specific ameloblasts have ceased to form enamel whereas others were partly or completely able to fulfil their task[5,6].

The aetiology behind enamel hypoplasia has been discussed in the literature, but no clear-cut aetiological factors seem to have been found. It is evident, however, that calcium homeostasis, during the period of mineralization of the teeth, has some bearing on the occurrence of enamel hypoplasia[7]. Other studies have not shown a significant association between enamel defects and calcium deficiencies[7–10]. The connection between diseases and trauma from, e.g., intubation in postnatal life, and the occurrence of enamel hypoplasia has been shown[11–13].

Enamel hypoplasia has been of interest in other research areas aside from dentistry. In anthropology, several articles have been published describing the frequency and patterns of dental enamel defects from historical populations[14–16] and among different primate populations[17–19]. Enamel hypoplasia is regarded as an indicator of episodes of systemic physiological stress during the period of tooth formation[20].

The morphology of the prisms and the prism pattern in teeth with enamel hypoplasia have been described from human and

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animal teeth[21–23]. Information is still limited on the microstructural level, therefore further studies are of importance to acquire a better understanding of the mechanisms behind enamel hypoplasia.

The aim of this study was to study the morphological appearance of enamel hypoplasia by means of polarized light microscopy and scanning electron microscopy in primary teeth.

Materials and methods

Nineteen exfoliated primary teeth (18 upper incisors, 1 molar), with clinically visible enamel hypoplasia were collected (Fig. 1). The inclusion criteria were teeth with a macroscopic defect of the enamel, involving the surface with a reduced enamel thickness and with rounded, smooth borders. All teeth were stored in saline with thymol until preparation. Each tooth was cut sagitally, in a bucco-lingual direction into halves, through the defect, with a Leitz low-speed saw microtome. Seventeen of the teeth (16 upper incisors, 1 molar) were embedded in an epoxy resin (Epofix®; Electron Microscopy Sciences, Fort Washington, PA, USA) and serially sectioned longitudinally in a bucco-lingual direction, with a Leitz low-speed saw microtome, to a thickness of about 120 μm. The sectioning always aimed to leave a central section for further analyses. Two primary incisors were cut into two halves.

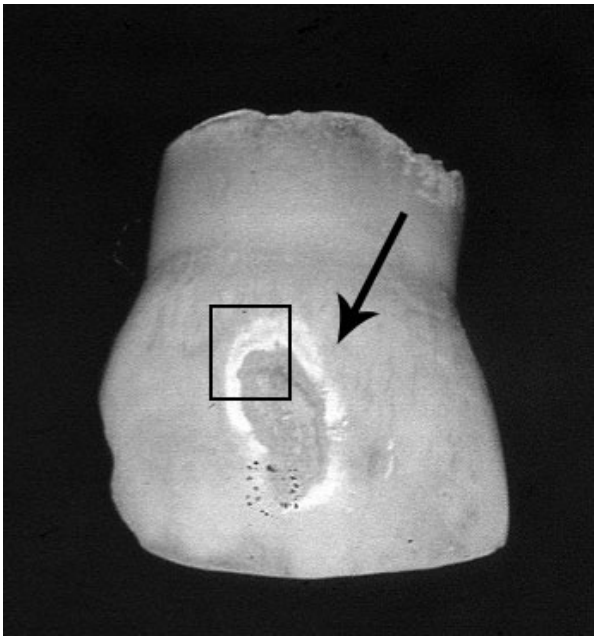

Fig. 1. Macro photo of an upper central incisor with a buccal enamel hypoplasia (arrow). The square marks location for the SEM images.

All sections were examined dry in air and after water imbibition in a polarized light microscope (POLMI).

All sections, including the exterior and the cut surface of the two tooth halves were etched for 30 s with 30% phosphoric acid and carefully rinsed with de-ionized water. The time for etching was based on previous own studies in scanning electron microscope (SEM) and other studies. They were then mounted on sample holders for the SEM, sputter coated with 15–20 nm thick gold, and examined in a Philips SEM 515 at 20 kV (Philips, Eindhoven, the Netherlands) and a field emission SEM (Gemini IMB, LEO 1530; Oberkochen, Germany).

Ethical considerations

All teeth analysed in this study were donated by the patients during a period of several years, when no consent from the local ethical committee was necessary for the collection and study of exfoliated primary teeth. None of the teeth could be, however, traced to a specified individual and the handling of the tooth material was carried out with full ethical respect for any patient.

Results

Despite, in some cases, a long storage time for the teeth, no morphological changes were noted that could be attributed to storage *per se*. All enamel hypoplasias had the same general morphological appearance when examined in a POLMI or SEM. Only minor differences were found between the specimens.

Polarized light microscopy

The enamel hypoplasia had, at both the cervical and the incisal margins, rounded borders with the exception for those teeth that were

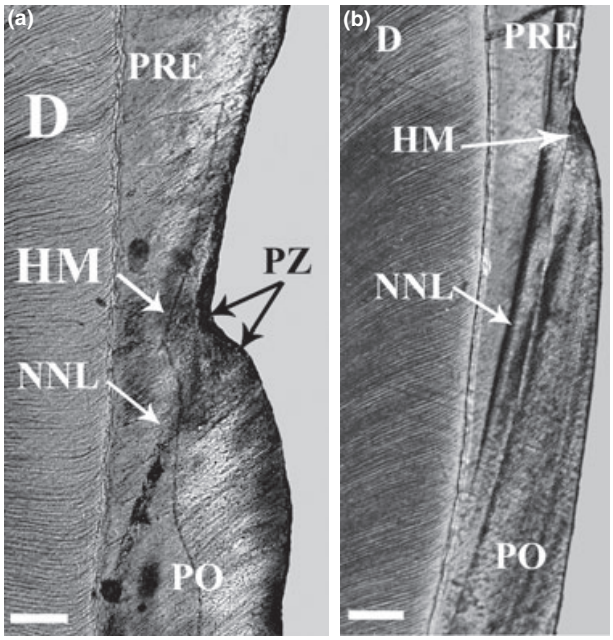

Fig. 2. Un-decalcified sections of primary incisors with enamel hypoplasia (original magnification 40×): (a) enamel hypoplasia of neonatal origin; (b) enamel hypoplasia of postnatal origin. D, dentin; NNL, neonatal line; PRE, prenatal enamel; PO, postnatal enamel; HM, hypomineralized enamel; PZ, porous zone (white bar ~500 μm).

incisally abraded, and thus the coronal border could not be seen. There were no morphological differences found between enamel hypoplasia in the incisors and the primary molar. The enamel in the area of the enamel hypoplasia in the primary teeth appeared negatively birefringent. The base of the defect followed the direction of the neonatal line (Fig. 2a,b). In one of the sections, three areas of enamel hypoplasia were seen, all in relation to distinct postnatal incremental lines. The incremental lines, including the neonatal line, appeared positively birefringent as seen dry in air in polarized light.

The base of the enamel hypoplasia, showing no sign of a normal aprismatic surface layer, revealed a rough surface. No other aberrations were found with the exception for a reduction of the enamel thickness. In the area for the entrance of the incremental line, however, a more porous zone could be discerned (Fig. 2a). This zone appeared positively birefringent in polarized light that changed to negatively birefringence after water imbibition, indicating a pore volume distribution of <5%. In teeth with shallow enamel hypoplasia, macroscopically

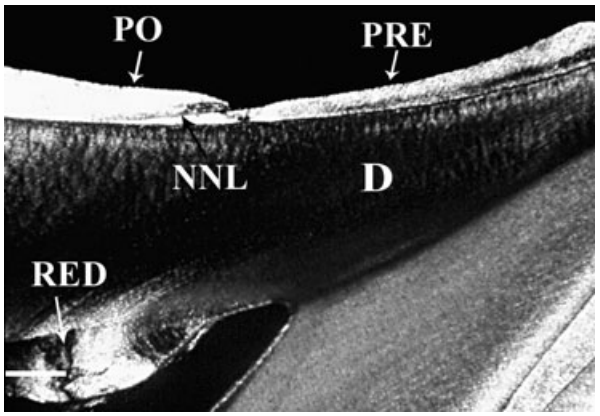

Fig. 3. Un-decalcified section of a primary incisor with a palatinal enamel hypoplasia and reparative dentin in the pulp. D, dentin; NNL, neonatal line; PRE, prenatal enamel; PO, postnatal enamel; RED, reparative dentin (original magnification 40×; white bar ~1 mm).

seen as a band on the tooth surface, it was surrounded by normal enamel.

Corresponding to the porous base of the enamel hypoplasia, reparative dentin was found in the pulp. In one of the specimens with an area with enamel hypoplasia located on the lingual surface, a more extensive zone of reparative dentin had been formed (Fig. 3).

The cervical and the incisal borders of the enamel hypoplasia had a rounded appearance due to the prisms in the non-affected enamel being bent, which may be attributed to a change of the prism direction.

In none of the specimens could caries be found.

Scanning electron microscopy

In low magnifications, the rounded borders and the rough surface of the base of the hypoplasia were readily seen (Fig. 4). The rounded borders had a normal surface structure whereas the base of the defects appeared rough and porous and the etched prism ends were seen (Fig. 5a). Between the base of the defect and the rounded cervical border, a hypomineralized area was noted with less densely packed prisms with an irregular appearance (Fig. 5b).

When the un-decalcified sections were examined, a distinct rupture was seen close to the area of the hypoplasia corresponding to the location of the neonatal line. The prenatally formed prisms had concave endings

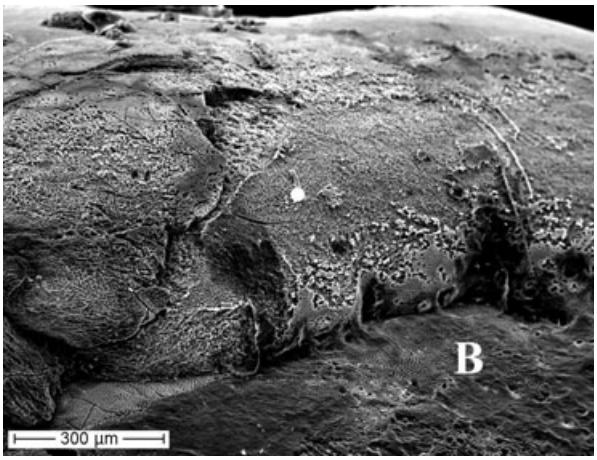

Fig. 4. SEM image of the enamel surface with an enamel hypoplasia showing the rounded cervical border and the rough surface of the base of the defect (B, base of the defect; magnification 100×; bar 300 µm).

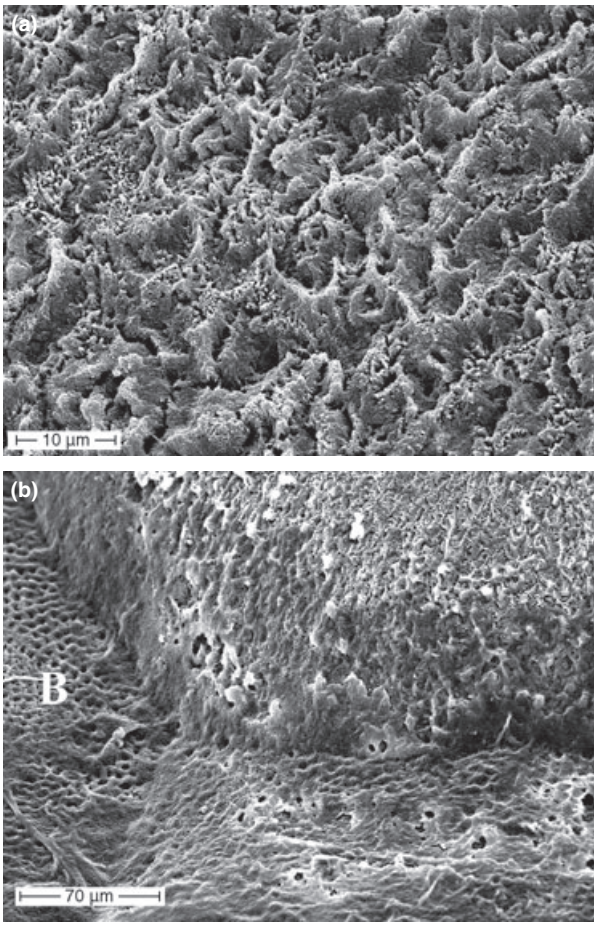

Fig. 5. SEM ages of enamel hypoplasias. (a) Base of the defect with irregular and rough prism ends (magnification 2000×; bar 10 µm). (b) The junction between the base and the cervical border of an enamel hypoplasia with porous enamel (B, base of the defect; magnification 400×; bar 75 µm).

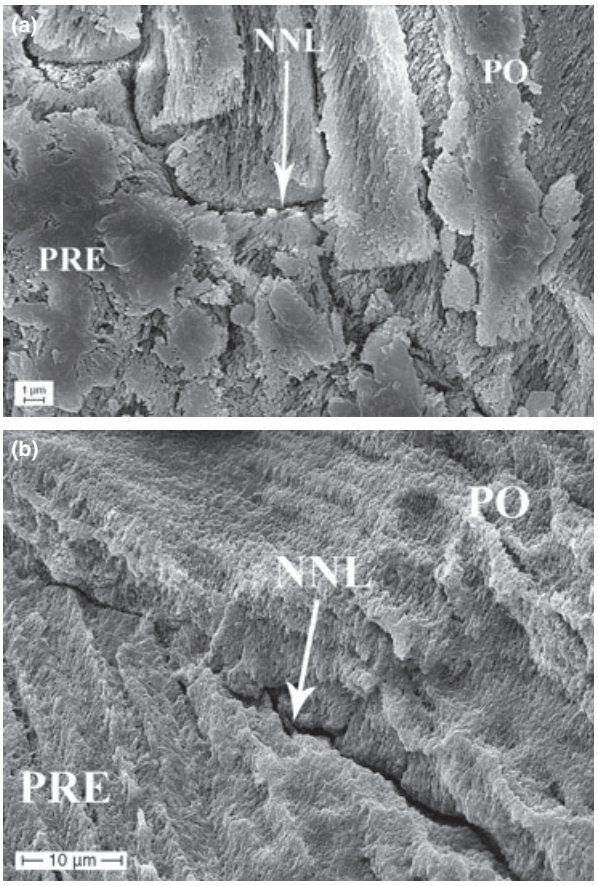

Fig. 6. SEM images of un-decalcified sections of primary incisors, the locations are marked in Fig. 1. (a) Concave prenatal and convex postnatal prism endings (magnification 4000×; bar 1 µm). (b) SEM image showing the change in prism direction (magnification 2000×; bar 10 µm). NNL, neonatal line; PRE, prenatal enamel; PO, postnatal enamel.

whereas the prisms formed postnatally had a convex ending (Fig. 6a).

Discussion

This study has shown that primary teeth with enamel hypoplasia have not only minor morphological changes, but also a reduced enamel thickness, with an incomplete enamel mineralization in the base of the defect.

The teeth in this study were collected over a long period of time; however, they were all stored under the same conditions. Therefore, there is no reason to believe that any morphological differences or changes may be attributed to the storage time or the storage medium. Further, the findings of the morphological appearance did not differ between teeth with different storage time.

The histo-morphological appearance of the enamel hypoplasia, seen in the polarized light microscopy, has previously been observed and was explained as a localized disruption of the amelogenesis resulting in an enamel defect[8]. Yet, reparative dentin was found in one section as a possible reaction to the porous prenatal enamel.

The macroscopic defect in an enamel hypoplasia is formed along an incremental line, in most of the present cases the neonatal line. It has been proposed that the ameloblasts are very sensitive to disturbances during their early maturation stage and do not recover easily[6]. It must be remembered that enamel mineralization progresses on several fronts; however, all enamel may be in the maturation stage at the same time[24], which may explain the wide extension of nonlinear enamel hypoplasia and the lack of a normal surface with an underlying porous enamel. Other important factors that have to be considered are ameloblastic activity and the severity and duration of the insult[25].

A bending of the prisms in the rounded borders was observed both in POLMI and SEM. As the ameloblasts in the area of the enamel hypoplasia have not produced any more enamel, the un-affected ameloblasts, having no neighbouring ameloblasts, have reacted by successively forming a rounded border. The finding of changes in the prism directions has been noted previously[3,23]. A possible reason may also be that the ameloblasts in the cervical border were in a secretion stage and, whatever the reason for the disturbance, the ameloblasts alter their physiochemical conditions for maturation[6]. It has been suggested that the sensitivity of the ameloblasts depends on their stage of development, thus leading to different modes of reaction[6]. This supports the histological findings of few enamel aberrations, but the reduction of the enamel thickness and hypomineralized character of the enamel in the cervical border. Of special interest were the shallow enamel hypoplasias as the surrounding enamel appeared normal. This may imply that if the time for the occurrence of an enamel hypoplasia is late in the enamel formation, the surrounding ameloblasts apparently are not affected.

In many respects, the SEM findings coincide with the POLMI findings with more porous enamel at the base of the defect and change of prism direction in the cervical area. Ultra structural studies of enamel with hypoplasia using atomic force microscopy confirm the porous surface with smaller crystallites than normal, which indicate a lack of maturation[22]. The SEM appearance is in accordance with what has been found in several other studies of both human and primate teeth[3,14,15,21,23,26]. In a SEM study of the development of rat incisor enamel hypoplasia, it was shown that the stage of transmission of the ameloblasts from secretion to maturation was affected, which is similar to the findings here[27].

The morphological appearance of enamel hypoplasia clearly suggests that ameloblasts in certain stages are affected in different ways. Different medical conditions may, however, pre-dispose the ameloblasts to become more sensitive due to an underlying hypocalcaemia, which is found in connection with different conditions during the neonatal and postnatal period. Hypoplastic lesions in sheep incisors may be produced by local and systemic means, which is not a contradictory to the present findings[3,23,28].

Previous studies have shown a significant association between enamel hypoplasia and caries[29,30]. Even though no caries could be detected in the examined samples in this study, enamel hypoplasia, with its porous and rough base surface, increases the risk for caries. Further, the porous enamel constitutes a pathway for bacteria and other stimuli that may affect the pulp, as seen as formation of reparative dentin in the pulp.

Conclusions

The morphological appearance of enamel hypoplasia with rounded borders and a porous bottom indicates that only certain ameloblasts are affected. The porous bottom constitutes possible pathways for bacteria into the dentin.

What this paper adds

- This study adds information about the morphology of enamel hypoplasia in primary teeth.
- The rounded borders of enamel hypoplasia may be explained by a bending of the enamel prisms.
- The bottom of enamel hypoplasia is rough and more porous.

Why this paper is important to paediatric dentists

- It improves the understanding of the mechanisms behind enamel hypoplasia in primary teeth.

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