International Journal of Paediatric Dentistry 2005; **15:** 233–240

Scanning electron micrograph analysis of hypomineralized enamel in permanent first molars

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Summary. First molars with cream- to yellow-coloured demarcated opacities of the enamel, often in combination with severe loss of substance, are common in many child populations. The aetiology is obscure.

Aim and Method. The aim of this study was to study the ultrastructure of the enamel of 10 affected teeth by means of scanning electron microscopy (SEM) in order to gain a better understanding of the clinical appearance and treatment problems of this condition, and to find some clues to its aetiology.

Results. The basic enamel structure with enamel rods and interrod zones was found in porous parts of the enamel, as well as in normal parts, but the packing of the hydroxylapatite crystals seemed to be looser and less well organized in the porous parts. The border between normal and hypomineralized enamel was usually distinct, and followed the direction of the rods. The preserved basic structure indicates normal function of the ameloblasts during their secretion phase, but impaired function during their maturation stage.

Conclusion. Considering the poor etch profile, it seems reasonable to recommend removal of all affected enamel surrounding the cavity, if possible, and to use a glass ionomer filling with its chemical bonding to tooth substrate, when restoring first molars with remaining affected enamel.

Introduction

The developmental dental defects of enamel can be classified as quantitative enamel hypoplasia or qualitative enamel hypomineralization. Hypomineralized enamel can be seen clinically as either demarcated defects or diffuse opacities, which are typical for fluorosis. Well-mineralized enamel is translucent, while hypomineralized enamel shows abnormal translucency, depending on the increased pore volume distribution in the tissue [1]. Variations in colour from white to yellow or brown can be seen. Hypomineralized enamel exhibit lower hardness values compared to normal enamel [2].

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First molars with developmental enamel defects are a common finding (4·4–19·3%) in many child populations [3–6]. This condition has been termed molar-incisor-hypomineralization (MIH) [7,8]. The colour of the defects can range from whitish yellow to yellow-brown. Furthermore, they can appear as demarcated opacities to severely broken enamel (Fig. 1a–d). Clinical studies from Sweden and Finland have shown that as many as 6·5–8·4% of children can have severe defects with disintegration of the enamel [4,5]. Incisors can also be affected concomitantly with the lesions in permanent first molars (Fig. 1d). When several molars are affected, the relative risk of incisors showing opacities is increased [4,6], and when a patient has a severe defect, it is likely that the contralateral tooth is also affected [4,9]. These findings indicate that the origin is caused by a systemic factor during the first year of life.

Fig. 1. Teeth with molar-incisor-hypomineralization (MIH): (a) A maxillary first molar with opaque and discoloured mesial, occlusal and palatal areas. (b) A mandibular first molar with opaque and discoloured mesial, occlusal, distal and lingual areas with posteruptive enamel loss and cavity formation. (c) A mandibular first molar which has been restored because of extensive hypomineralization of its occlusal parts. There is continued disintegration at the margins of the filling. (d) Large demarcated opacities of the upper central incisors in a child affected by MIH. Note the loss of substance at the incisal edge of right central incisor.

Several aetiological factors have been connected to these defects, such as oxygen shortage of the ameloblasts, dioxin in breast milk, respiratory diseases and environmental changes [3,9–13]. It is evident that further studies are warranted to clarify the aetiological factors and mechanisms behind the defects, and to create proper treatment strategies.

With the exception of enamel alterations in hereditary conditions such as amelogenesis imperfecta [14,15] and epidemiolysis bullosa [16], or in conditions caused by known aetiological agents such as fluoride [17–19], few studies [2,20] have dealt with the histomorphology behind hypomineralized enamel and its characteristics. However, the morphological appearance of the enamel of permanent first molars with demarcated opacities has been investigated by polarization microscopy [21]. Secondary ion mass spectrometry and X-ray micro-analysis [22] has revealed a higher carbon content and a lower calcium content, as well as phosphorous in the hypomineralized enamel of MIH enamel. The average Ca/P ratio of 1·4 is significantly lower in hypomineralized areas compared with normal enamel.

The aim of this study was to investigate the ultrastructure of hypomineralized enamel from first permanent molars by means of scanning electron microscopy (SEM).

Materials and methods

Immediately after extraction, the MIH molars were put in buffered 4% formaldehyde and stored for 24 h in ethanol prior to embedding in an epoxy resin. They were cut sagittally in two halves in a bucco-lingual direction with a Leitz low-speed saw microtome [23]. A central section with a thickness of 120 µm was then prepared from one half of each tooth in the saw microtome. All central sections were examined in polarized light microscope [21]. A total of 73 molars were examined.

The teeth investigated in this study have been described previously in a morphological study [21]. For convenience, a short résumé of the histological findings is presented here. The hypomineralized areas extended cervically from the cusps, compromising about half of the buccal and/or lingual sides. In all cases, the enamel of the cervical area was well mineralized. The borders between the hypomineralized and normal enamel were distinct and followed the lines of Hunter-Schregers. The opacities showed a varying degree of porosity, the yellow-brown defects were more porous than the white-cream ones and extended through the whole enamel layer, while the white-cream opacities were situated in the inner parts of the enamel.

Scanning electron microscopy

Ten teeth with clinical and histological characteristics of MIH were selected for SEM examination. One of the tooth halves was etched with 30% phosphoric acid for 30 s, carefully rinsed with distilled water, mounted on sample holders for the microscope and sputter coated with gold. The cut surfaces as well as the natural surfaces were investigated in a Philips SEM 515 at 15 kV.

Microradiography

Contact microradiographs of the central sections were made in a Philips micro X-ray machine, exposing Kodak spectrographic Plate 069 to nickel-filtered copper radiation excited at 20 kV and 20 mA. The microradiographs were examined under transmitted light at low magnification.

Results

The enamel of the control teeth showed well-defined enamel rods with distinct borders and narrow interrod zones. The crystals, building up the enamel rods, were densely packed and their orientation was uniform and well organized (Fig. 2).

The enamel in normal areas of the MIH teeth showed the same morphological appearance as the control teeth (Fig. 3d).

In the porous areas, the enamel rods had a normal course and a normal basic morphology. However, a varying degree of decomposition of the structural pattern, depending on the degree of hypomineralization, could be seen. In moderately porous areas, the borders of the enamel rods were more or less blurred, and the interrod zones were not always distinguishable. The crystals seemed to be more loosely and irregularly packed (Fig. 3b). Very thin irregular enamel rods with wide interrod zones were seen in severely porous areas close to the surface and close to disintegrated enamel (Fig. 4b,c). The crystals were irregularly orientated and difficult to distinguish (Fig. 4c). The enamel surface was highly porous (Fig. 4d). In general, the hypomineralized enamel showed fewer distinct features compared with the normal enamel.

The border between the normal and hypomineralized areas following the rod direction was evident (Fig. 3c).

The microradiographs confirmed the hypomineralized character of the enamel in the disturbed areas (Figs 3a & 4a).

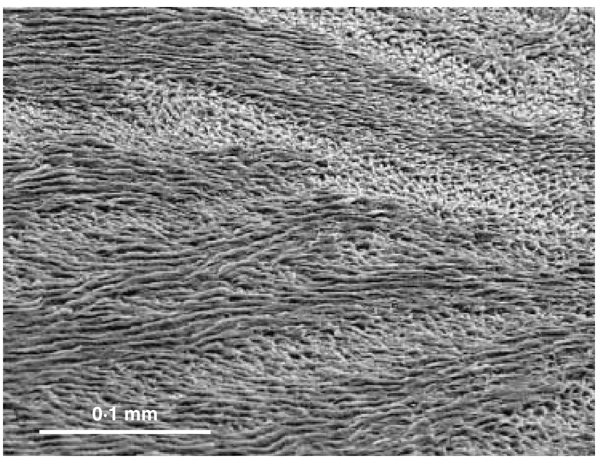

Fig. 2. Scanning electron micrograph of the cut surface of a normally mineralized permanent first molar.

Discussion

This SEM study showed that the basic enamel structure, with hydroxylapatite crystals forming rods and interrod enamel, was found in the porous zones as well as the well mineralized zones of the enamel. However, the packing of the crystals seemed to be less tight and less well organized in the porous parts. The borders of the enamel rods were indistinct and the interrods zones hardly visible, or the rods were very thin with wide interrod zones.

The featureless and amorphous appearance of porous enamel has been shown earlier in a SEM study of yellow demarcated enamel opacities [2]. It is obvious that etching by 30% phosphoric acid prior to the SEM analysis could not create a normal etch relief of the porous enamel. The reason for poor acid solubility could possibly be explained by a higher content of organic matter in the hypomineralized areas. This is supported by a previous study in which porous enamel had higher carbon content than normal enamel [22], indicating persistent carbonate or remnants of organic material. However, carbonate substitution into the hydroxyapatite lattice is known to increase the acid solubility of the mineral and would work to the opposite effect [24]. Thus, it can be presumed that the raised level of carbon depends on remnants of organic material, i.e. proteins. It has been suggested that protein might reduce access of inorganic ions to the crystallite surfaces [25].

Compared with the histology of early caries lesions, so-called 'white spots', changes in hypomineralized

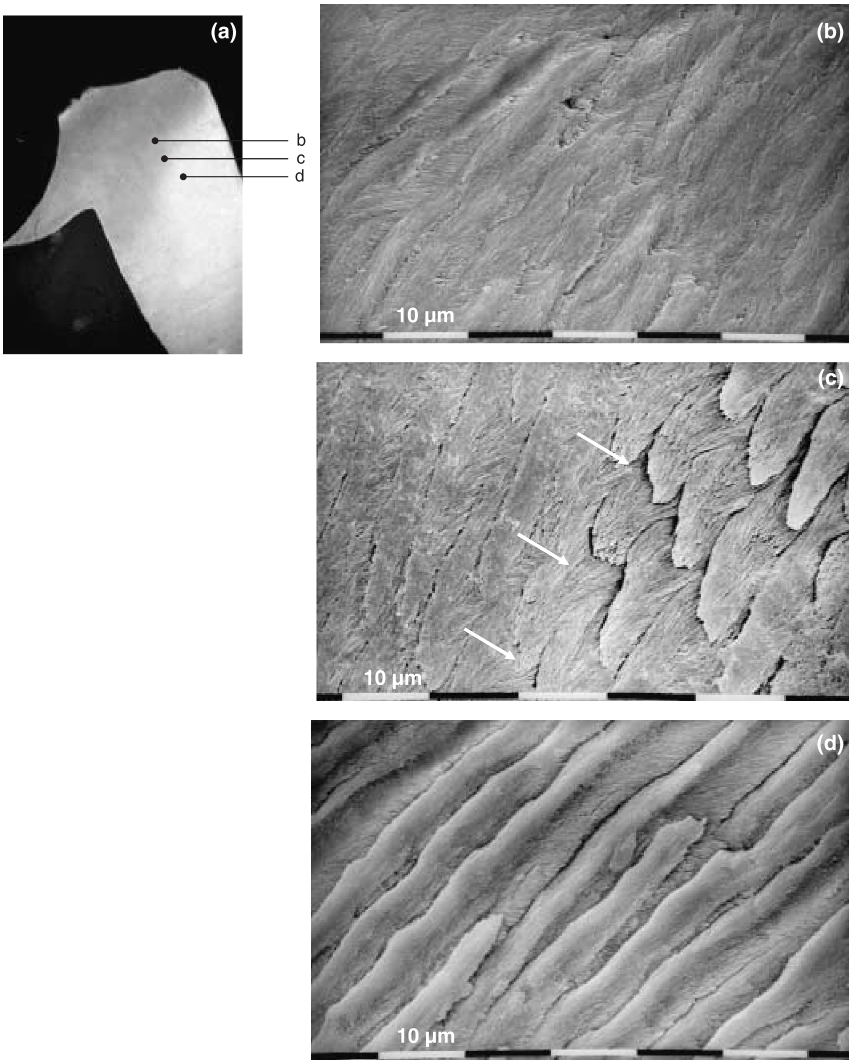

Fig. 3. Microradiograph and scaning electron micrographs (SEMs) of the cut surfaces from the cuspal parts of a tooth, where the porous area comprises the occlusal surface and part of the cusps: (a) Microradiograph confirming the hypomineralized character of the enamel in the disturbed area. The arrows show where the scans were performed. (b) A SEM of the porous area just medial to the border between the normal and hypomineralized areas. (c) A SEM demonstrating the border (see arrows) between the normal and hypomineralized areas. (d) A SEM of the normal mineralized enamel just lateral to the border between the normal and hypomineralized areas.

enamel and the body of the caries lesion had a similar appearance. However, in contrast to the examined demarcated opacities, with its porous zone extending from the surface to the dentin–enamel border mainly following the course of the prism, the 'white spot lesion' shows a wedge-shaped porous defect under the intact subsurface layer with the basis at the enamel surface [26]. Therefore, the dissolved appearance of the enamel prism and the interprismatic substance shown in Fig. 4b–d is thought to be a secondary cariogenic defect of originally porous zone. Acids produced by bacteria in the dental plaque most likely have excellent accesses to this basically hypomineralized enamel, especially in areas adjacent to disintegrated zones.

The enamel ultrastructure of different types of amelogenesis imperfecta (AI) has been investigated, but the appearance of the enamel seems to differ from that of demarcated opacities. In hypocalcified AI, the prisms have a normal course and morphology, but in contrast to demarcated opacities, there are also normal interprismatic spaces [15]. Hypomaturation type of AI has a discernible prismatic structure, but marked decussations of the prism were seen [27]. To the authors' knowledge, the unorganized and diffuse appearance of the enamel crystals shown in this study have never been demonstrated in any AI teeth.

The ultrastructural appearance of fluorotic enamel also differs from that of demarcated opacities. In cases of mild fluorosis, an increased porosity along the striae of Retzius can be seen. As the severity of fluorosis increases, the striae of Rezius will be more pronounced and there will be a continuous zone of

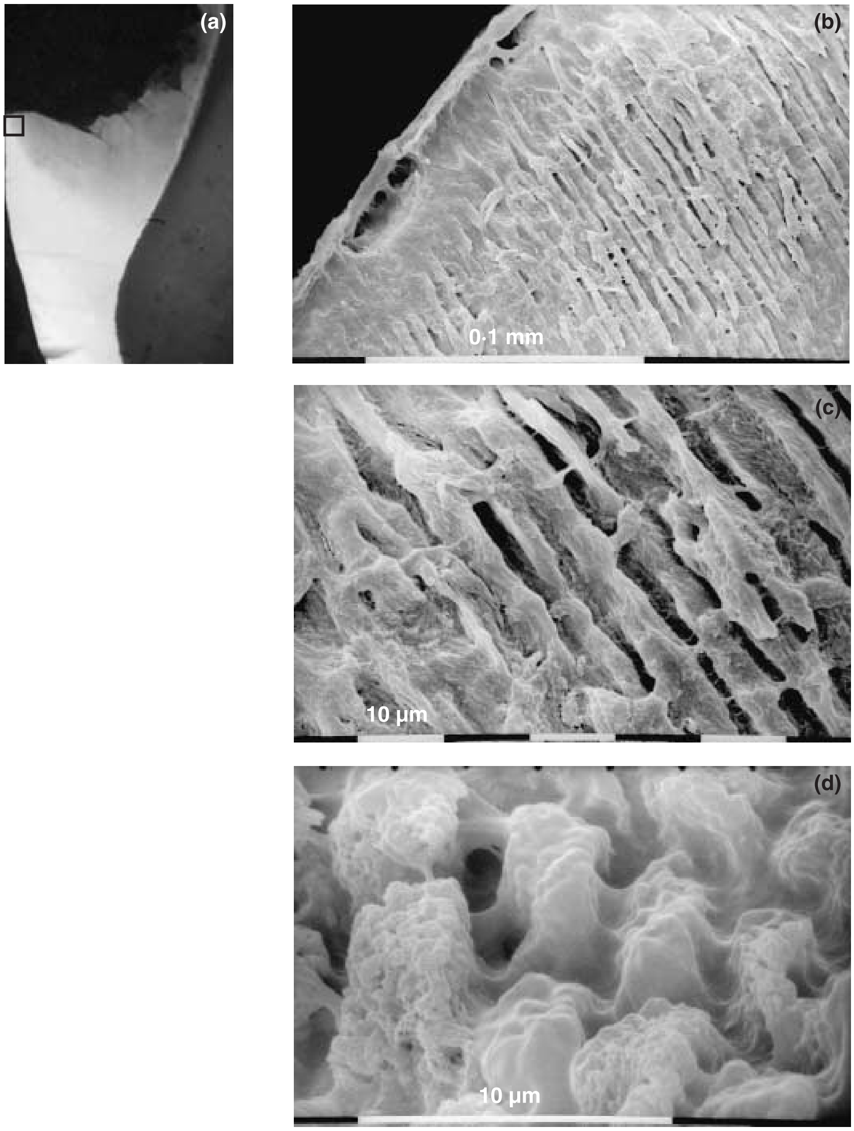

Fig. 4. Microradiograph and scaning electron micrographs (SEMs) of the cut surface from the cuspal parts of a tooth, where the porous area comprises the occlusal surface and the occlusal third of the lateral surfaces: (a) Microradiograph confirming the disintegration and the hypomineralized character of the enamel in the disturbed area. The square marks the area where the scans were performed. (b, c) Two SEMs performed at the cut surfaces close to the surface and close to the disintegrated enamel. (d) A SEM from the enamel surface of the same area demonstrating pronounced porosity.

porosity along the surface enamel. In porous zones, there are increased intracrystalline spaces both in rod and interrod enamel; this is particularly pronounced along the rod boundaries [18,19].

The enamel is formed during two main phases of ameloblastic activity, the formative secretory phase when the matrix is laid down and the maturation phase when the mineralization mainly occur.

The enamel crystals are laid down during the secretory stage, and the matrix architecture is probably the primary determinant for their orientation and growth. During the maturation stage, the crystals increase in width and thickness. The maturation can be subdivided into two or three phases [2,28]. Thus, the demonstrated impaired crystal orientation could be either an effect of disturbed ameloblastic activity during matrix secretion or maturation [21].

The well-defined border between the normal and porous areas running parallel to the rods (Figs 3a,c & 4a) shows that, even though a group of cells are impaired, the neighbouring cells have a normal function. This indicates that cells in a specific stage of development are affected [21].

Clinically, first molars with demarcated opacities and disintegration have been recognized to create problems when attempting to perform adequate filling therapy. There are retention problems, and also problems with marginal fractures of tooth structures as well as with fillings. With knowledge about the ultrastructural appearance of the porous enamel, it is easy to understand the problems surrounding the cavity, i.e. either very fragile enamel just about to collapse or enamel that cannot get an adequate etch profile to retain resin composite restorations. Therefore, it is reasonable to propose removal of as much affected enamel surrounding the cavity as possible when restoring MIH teeth [29]. Considering the poor etch profile, a glass ionomer filling, with its chemical bonding to tooth substrate [30], may be preferred when restoring hypomineralized first molars.

What this paper adds

- This paper describes the appearance of hypomineralised enamel derived from first permanent molars.
- Basic features of enamel structure, including enamel rods and interred zones were found in porous as well as normal parts of the enamel but the crystals were looser and less well organised.
- The border between porous and normal enamel was clear and well marked. Contrary to previous findings there was little clear difference in gingivitis.

Why this paper is important for paediatric dentists

- Demonstration of the structure of hypomineralised enamel in this paper helps paediatric dentists understand the problems founds in clinical treatment of patients with molar-incisor hypomineralisation.
- Difficulties in obtaining sufficient retention through etching or cavity preparation suggest that is reasonable to propose removing as much affected enamel as possible or to use additional methods to enhance bonding.

It has been suggested that pretreatment of enamel surface in hypomineralized AI with 5% sodium hypochlorite (NaOCl) prior to etching will result in improved enamel bonding [31]. NaOCl pretreatment might also enhance the bonding of MIH enamel.

Résumé. Les premières molaires avec opacités de l'émail de teinte crème à jaunâtre, souvent associées à des pertes sévères de l'émail, sont fréquentes dans de nombreuses populations d'enfants. L'étiologie est obscure. L'objectif de cette étude a été d'étudier l'ultrastructure de l'émail de 10 dents affectées à l'aide d'un microscope électronique à balayage (SEM).afin de mieux comprendre l'aspect clinique, les problèmes posés lors du traitement et d'obtenir des indices quant à l'étiologie. La structure de base de l'émail avec prismes de l'émail et zones interprismatiques a été retrouvée dans les zones poreuses de l'émail de même que dans les zones saines, mais l'assemblage des cristaux d'hydroxyapatite semblait plus lâche et moins bien organisé dans les parties poreuses. La limite entre l'émail normal et hypominéralisé était généralement distincte et suivait l'orientation des prismes. La préservation de la structure de base indique une fonction normale des améloblastes durant la phase de sécrétion mais une fonction altérée durant la phase de maturation.

Considérant le faible potentiel de mordançage, il semble raisonnable de recommander l'élimination, si possible, de tout émail affecté autour de la cavité ainsi que l'utilisation de verre ionomère, connu pour sa liaison chimique au substrat dentaire, lors de la restauration de premières molaires présentant cet émail atteint.

Zusammenfassung. Erste bleibende Molaren mit cremefarbenen oder gelblichen scharf begrenzten Opazitäten, häufig begleitet von ausgeprägtem Substanzverlust, sind in zahlreichen Populationen von Kindern verbreitet. Die Ätiologie ist unklar. Ziel dieser Studie war es, den Schmelz von 10 betroffenen Zähnen rasterelektronenmikroskopisch zu untersuchen, um Erkentnisse im Hinblick auf klinische Erscheinung und Behandlungsprobleme zu gewinnen sowie Rückschlüsse auf die Ätiologie zu ziehen. Die Grundstruktur des Schmelzes mit Schmelzprismen und interprismatischen Zonen war ebenso zu erkennen wie in normalem Schmelz, aber die Packungsdichte der Hydroxylapatitkristalle erschien geringer und weniger organisiert in den porösen Teilen. Der Verlauf der Grenze zwischen normalem und hypomineralisiertem Schmelz folgte gewöhnlich der Ausrichtung der Prismen. Die erhaltene Grundstruktur deutet auf eine normale Funktion der Ameloblasten während der Sekretionsphase aber eine gestörte Funktion während der Schmelzreifung hin. In Anbetracht des schwachen Ätzprofils erscheint es sinnvoll, die Entfernung allen an eine Präparation grenzenden betroffenen Schmelzes zu empfehlen, soweit dies möglich ist. Sofern erste bleibende Molaren zu versorgen sind mit verbleibendem defekten Schmelz am Präparationsrand ist auf Glasionomerzement mit dessen chemischer Adhäsion zurückzugreifen.

Resumen. En muchas poblaciones de niños son comunes los primeros molares con marcadas opacidades del esmalte que van desde el color crema al amarillo, a menudo combinadas con severa pérdida de sustancia. La etiología es obscura. El objetivo de este trabajo fue estudiar la ultraestructura del esmalte de 10 dientes afectados, por medio del microscopio electrónico de barrido (MEB) para mejorar el conocimiento sobre el aspecto clínico, los problemas de

tratamiento y conseguir algunas claves sobre su etiología. La estructura básica del esmalte con prismas y zonas interprismáticas se encontró en las partes porosas del esmalte así como en las partes normales, pero el relleno de cristales de hidroxiapatita parecía ser más suelto y menos bien organizado en las partes porosas. El límite entre el esmalte normal y el hipomineralizado era generalmente distinto y seguía la dirección de los prismas. La estructura básica preservada indica una función normal de los ameloblastos durante la fase de secreción pero una función alterada durante el estadío de maduración. Considerando el pobre perfil del grabado parece razonable recomendar la eliminación de todo el esmalte afectado que rodea la cavidad si es posible y usar un relleno de ionómero de vidrio por su unión química al sustrato dentario, cuando se restauran primeros molares con esmalte remanente afectado.

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