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Bacterial invasion of dentinal tubules beneath apparently intact but hypomineralized enamel in molar teeth with molar incisor hypomineralization

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Background. The most common problems for a patient with molar incisor hypomineralization (MIH) are the collapse of enamel and cavitations, loss of fillings, and secondary caries, but most of all, severe hypersensitivity.

Objective. The aim of this paper was therefore to histologically study possible bacterial invasion of dentinal tubules beneath apparently intact, but hypomineralized enamel in permanent molars with MIH.

Material and methods. Five extracted permanent first molars diagnosed with MIH were fixated, demineralized, and sagittally serially sectioned in a buccolingual direction in a microtome with a thickness of 4–5 μm. Sections were stained with a modified Brown and Benn staining for bacteria, unstained sections were analysed in field emission SEM.

Results. Stained sections from the cuspal areas, below the hypomineralized enamel, the staining indicated the presence of bacteria in the dentinal tubules. The HTX staining showed that the pulp in sections without any findings was normal and free from bacteria or infiltrates from inflammatory cells. In sections where bacteria were found in the cuspal areas or deeper in the dentin, a zone of reparative dentin was found, and in sections from one tooth, the coronal pulp showed an inflammatory reaction with inflammatory cells. In sections adjacent to those without any bacterial staining, the SEM analyses revealed empty dentinal tubules without any odontoblast processes or signs of bacteria. When odontoblast processes were found, the dentinal tubules were filled with bacteria located on the surface of the odontoblast processes. In some areas, a large number of tubules were found with bacteria. No bacteria were found close to the pulp. The odontoblast processes appeared larger in areas where bacteria were found.

Conclusions. The presence of bacteria in the dentinal tubules and inflammatory reactions in the pulp indicate that oral bacteria may penetrate through the hypomineralized enamel into the dentin, thus possibly contribute to hypersensitivity of teeth with MIH.

Introduction

A high prevalence of molar incisor hypomineralization (MIH), seen as demarcated opacities in the enamel with beige to yellow brownish colour, has been described in literature[1–7]. Histological studies have revealed that MIH may be related to the first year of life[8]. The aetiology, however, is still not fully understood[9–11].

The most common problems for a patient with MIH are the collapse of enamel and cavitations, loss of fillings, and secondary caries, but most of all, severe hypersensitivity often resulting in severe discomfort and behaviour management problems and even dental fear[12]. The reason for hypersensitivity, however, is still not fully understood. In severe cases, extraction of the affected molars may be the therapy of choice. A recent article has shown that there are few problems concerning space closure and the development of the permanent dentition[13].

It is a well-established fact that bacteria may invade dentin tubules when a caries attack

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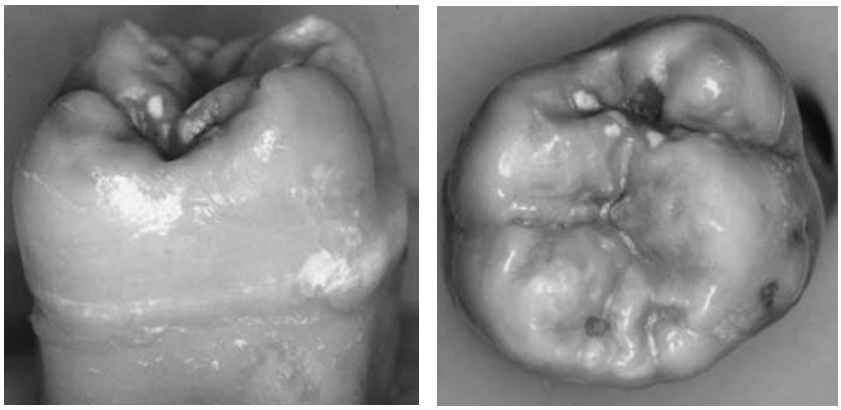

Fig. 1. Macro-photo of a permanent first molar diagnosed molar incisor hypomineralization analysed in this study.

is present, thereby causing a reaction in the pulp[14]. Even though oral bacteria may penetrate into non-carious dentin, there must be a pathway through the enamel, that is, a fissure or a crack. The highly microporous enamel in the more severe cases of MIH may very well constitute possible ways of transport through the enamel. Because MIH is an enamel aberration which is seen at the time of eruption of the first molars, the dentin tubules are still wide in which bacteria could easily penetrate. Thus, any penetration of bacteria or their metabolites may rapidly cause a pulp reaction, which is recognized by clinicians as a pulpitis. However, there are no histological studies of the dentin beneath hypomineralized enamel in young, permanent teeth.

The aim of this paper was therefore to histologically study possible bacterial invasion of dentinal tubules beneath apparently intact, but hypomineralized enamel in permanent molars with MIH, in demineralized stained sections, using light microscope and scanning electron microscope (SEM).

Materials and methods

Tooth samples and preparation

Five extracted permanent first molars with the diagnosed MIH were fixated in 10% neutral buffered formalin for at least 48 h. Macro-photos were made from all of the teeth from different views in order to relate any histological findings with the clinical appearance of the teeth (Fig. 1).

Demineralization of the teeth was carried out in formic acid/sodium citrate (22.5%) for 1 month. The demineralization was controlled by radiography. The specimens were embedded in paraffin blocks and sagittally serially sectioned in a bucco-lingual direction in a microtome, with a thickness of 4–5 μm.

Every third section was stained with haematoxylin and eosin (HTX), and every adjacent section was stained with a modified Brown and Brenn (B&B) technique for staining of bacteria[15], thus leaving every third section unstained. The stained sections were covered with cover glass and examined under a light microscope.

Selected unstained sections, adjacent to those sections where bacteria were found in the light microscope, were cleaned from paraffin for the SEM analyses. Five sections, stained with the modified B&B technique, were used for the SEM analysis after removing the cover glasses with Zylen.

SEM analysis

All specimens were sputter coated with gold prior to the SEM examination, which was carried out in a field emission SEM (Gemini IMB, LEO 1530, Oberkochen, Germany) at 10 kV. Specimens examined with backscattered electrons were sputter coated with a thin layer of carbon.

Results

Light microscopic findings

Bacteria staining. In sections from the cuspal areas below the hypomineralized enamel, the dentinal tubules were stained, indicating the presence of bacteria (Fig. 2a). The bluish staining

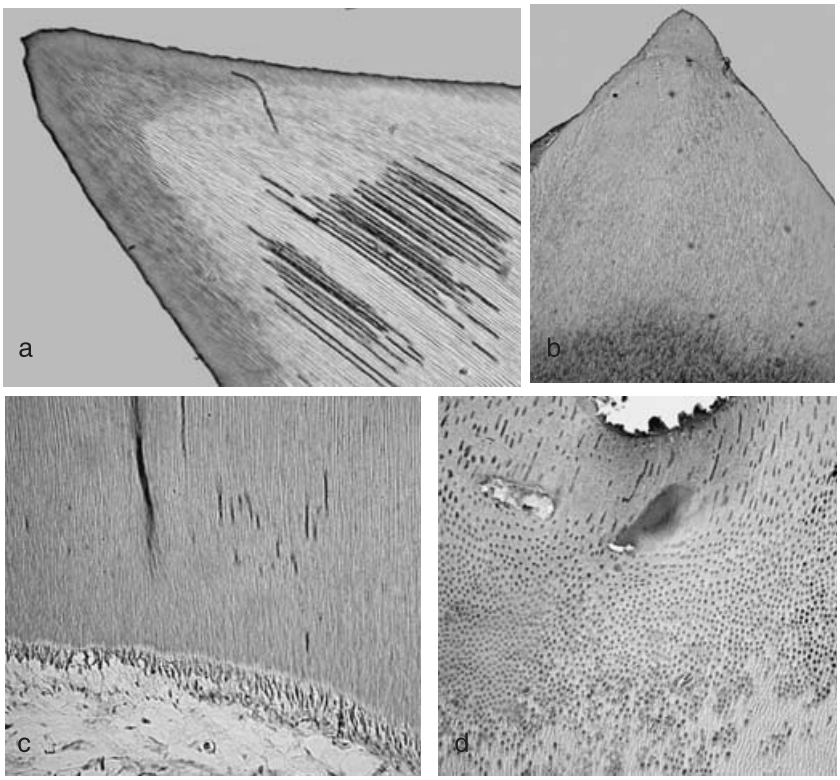

Fig. 2. Demineralized sections from permanent first molars diagnosed with molar incisor hypomineralization, stained with the modified Brown and Brenn technique. (a) Staining of bacteria in the cuspal parts of the dentin deep to hypomineralized enamel (magnification ×40). (b) Section deep-to-normal enamel without any stained bacteria (magnification ×40). (c) Stained bacteria in the dentin close to the pulp (magnification ×40). (d) Stained bacteria in carious dentin deep to the occlusal surface (magnification ×40).

indicates that the bacteria were Gram-positive. In some sections, the proximal parts of the dentin cusp appeared with the tubules, filled with bacteria. The bacteria were always found under the mantle dentin. In deep-to-normal enamel sections, no bacteria could be seen (Fig. 2b). This indicates that bacteria may penetrate through hypomineralized enamel into the dentin. In one specimen, bacterial staining could be seen close to the pulp (Fig. 2c).

One of the specimens had an occlusal caries attack penetrating deep into the dentin. A massive disintegration of the dentin was noted with bacteria staining in the tubules. In the occlusal parts of the carious dentin, the staining was more massive and even seen in conglomerates (Fig. 2d). The pulp was also affected, and stained bacteria were found in the pulp.

HTX staining. The HTX staining showed that the pulp, in sections without any findings of bacteria in the adjacent B&B-stained sections, was normal and free from bacteria or infiltrates from inflammatory cells (Fig. 3a). In sections where bacteria were found in the cuspal areas or deeper in the dentin, a zone of reparative dentin was found (Fig. 3b). In sections from one tooth, the coronal pulp showed an inflammatory reaction with inflammatory cells (Fig. 3c,d).

SEM findings

In sections adjacent to those without any bacterial staining, the SEM analysis revealed empty dentinal tubules without any odontoblast processes or signs of bacteria (Fig. 4a). Closer to the pulp, odontoblast processes were more frequently seen (Fig. 4b).

In sections adjacent to stained sections, bacteria were found on the surface of sections in the cuspal parts (Fig. 4c). As in the non-infected sections, few odontoblast processes were seen. However, when odontoblast processes were found, the dentinal tubules were filled with bacteria located on the surface and a destruction of the processes was also seen (Fig. 4d–f). In some areas, a large number of tubules were found with bacteria (Fig. 4g). No bacteria were found closer to the pulp. In the sections with carious dentin, a destruction

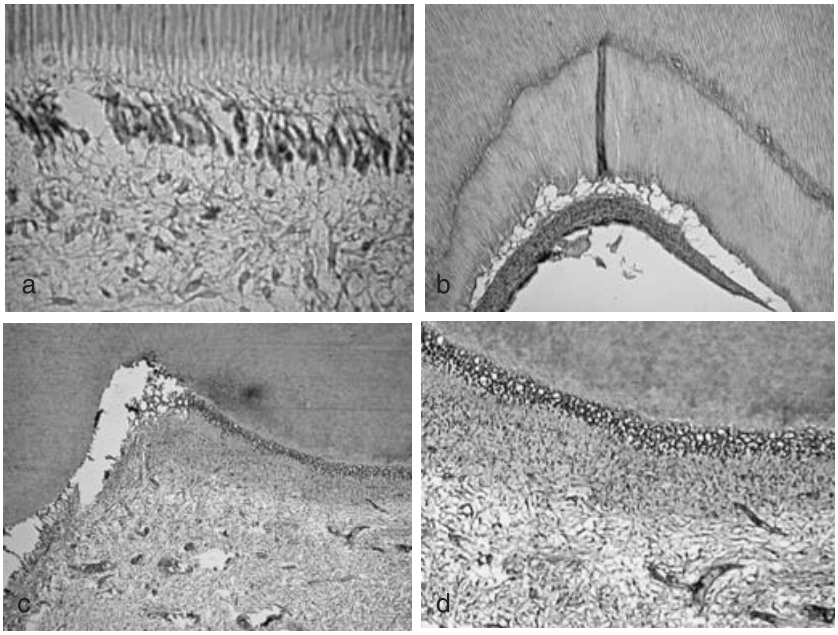

Fig. 3. Demineralized sections from permanent first molars diagnosed molar incisor hypomineralization, stained with haematoxylin and eosin. (a) Normal pulp without any bacteria or inflammatory cells (magnification ×40). (b) A zone of reparative dentin in the pulp deep to cuspal dentin with bacterial staining (magnification ×40). (c) Inflammatory reaction in the pulp deep to cuspal dentin with bacterial staining (magnification ×40). (d) Inflammatory reaction in the pulp deep to cuspal dentin with bacterial staining (magnification ×100).

of the dentin was seen and huge amounts of bacteria filled the dentin tubules (Fig. 4h).

In the backscatter mode, there was no difference in contrast between the odontoblast processes and the bacteria, which thus confirmed that both structures had the same basic organic structure (Fig. 5). Further, the demineralized dentin appeared more porous than seen in conventional SEM.

The B&B-stained sections, which in the light microscope were examined with backscattered electrons, revealed no presence of bacteria in areas with staining. The open tubules revealed no bacteria; however, the possibility of bacteria in non-open tubules may not be excluded. Nevertheless, bacteria were found deeper into the dentin as in the other samples (Fig. 6a,b).

The odontoblast processes appeared larger in areas where bacteria were found than in areas not invaded by bacteria (Fig. 7). Measurements in SEM of the affected processes revealed that they had a diameter twice as large compared with normal odontoblast processes.

Discussion

This study has shown that bacteria may appear in the dentinal tubules in the cuspal parts of teeth with MIH with apparently intact enamel. Further, little changes or reactions in the pulp were found.

Even though the number of teeth is limited in this study, the results clearly indicate that bacteria may penetrate through hypomineralized enamel with a macroscopically intact surface into the dentin. The bacteria penetration was always found deep in clinically observed hypomineralized enamel. Because the dentinal tubules in young, permanent teeth are wide, bacterial penetration into the tubules is not limited by intratubular mineralization and obliteration as seen in older dentin[16].

The validity of the B&B staining method has been discussed because it has shortcomings and is insensitive[17].

In the SEM analysis of demineralized sections, only the surface may be studied, therefore, only bacteria in tubules or lying on the surface may be seen. Few odontoblast processes and bacteria were found in the coronal parts of the specimens as seen in the B&B staining, even after analyses of the stained sections. The question still remains: what does B&B staining represent in coronal parts. The findings of bacteria on the surface of sections in the coronal parts may be attributed to an artifact from cutting in the sledge microtome or other stages in the preparation, resulting in a contamination of the surface.

Odontoblast processes, extend to the coronal parts of the dentin and not only in the inner third of the dentin, have been disputed for

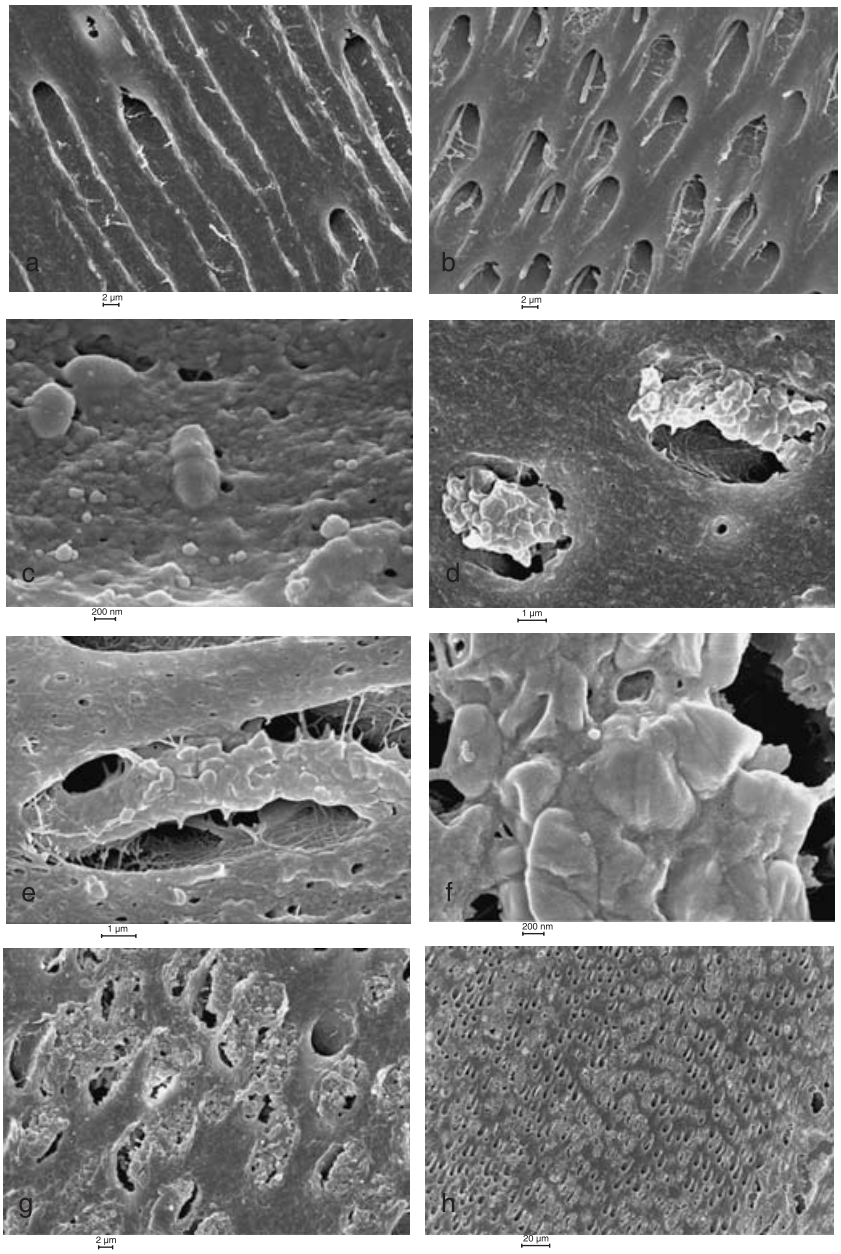

Fig. 4. Demineralized sections from permanent first molars diagnosed with molar incisor hypomineralization examined in scanning electron microscope. (a) Empty dentinal tubules without any odontoblast processes or signs of bacteria (magnification ×3200). (b) Dentinal tubules with odontoblast processes (magnification ×2000). (c) Bacteria found on the surface of the sections in the cuspal parts of the tooth (magnification ×30 000). (d) Dentinal tubules filled with bacteria located on the surface of the odontoblast processes and destruction of the processes (magnification ×8000). (e) Dentinal tubules filled with bacteria located on the surface of the odontoblast processes and destruction of the processes (magnification ×10 000). (f) Dentinal tubules filled with bacteria located on the surface of the odontoblast processes and destruction of the processes (magnification ×30 000). (g) Dentinal tubules filled with bacteria located on the surface of the odontoblast processes and destruction of the processes (magnification ×2000). (h) Dentinal tubules in carious dentin filled with bacteria located on the surface of the odontoblast processes and destruction of the dentin (magnification ×400).

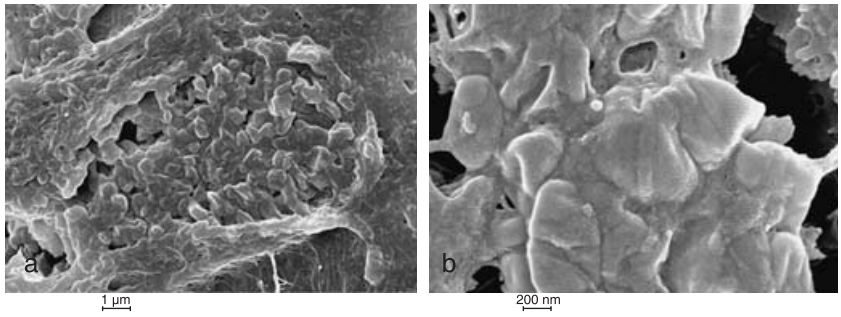

Fig. 5. Demineralized sections from permanent first molars diagnosed with molar incisor hypomineralization examined in scanning electron microscope with backscattered electrons. (a) Dentinal tubule with an odontoblast processes covered with bacteria (magnification ×8000). (b) Odontoblast process covered with bacteria (magnification ×30 000).

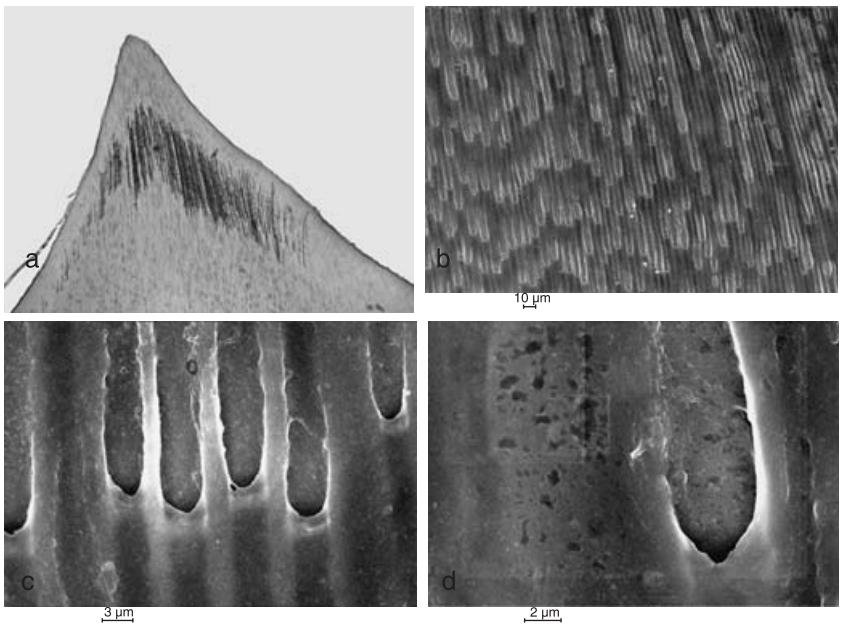

Fig. 6. Coronal part of demineralized dentin section examined in light microscope and scanning electron microscope with backscattered electrons. (a) Section stained according to Brown & Brenn. Coronal tubules stain indicating the presence of bacteria (magnification ×40). (b) Empty dentinal tubules in the same section as above taken in the stained area (magnification ×300). (c) Empty dentinal tubules in higher magnification indicating tubules deep to the open tubules (magnification ×2760). (d) Empty dentinal tubules showing porous structure in the dentin (magnification ×5000).

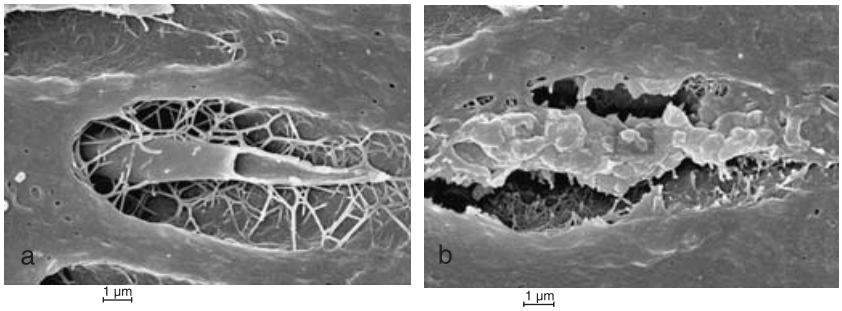

Fig. 7. Sections of demineralized dentin with dentinal tubules with odontoblast process examined in scanning electron microscope. (a) Odontoblast process without bacteria (magnification ×8000). (b) Odontoblast process covered with bacteria (magnification ×8000).

a long time[18–23]. In a study of human dentin, where the odontoblast processes were revealed by fluorescence labelling and transmission electron microscopy, the authors 'suggest that the odontoblast processes do not extend beyond the inner dentin of fully erupted human premolars'[24]. Even though there is evidence, *pros* and *cons*, of how far the processes actually extend, it is reasonable to believe that the few odontoblast processes found in the coronal parts of the dentin, in this study, can be explained by their limited extension.

The SEM analyses revealed that the bacteria were mainly attached to the odontoblast processes rather than to the walls of the tubules. It has been shown that dentin invasion of streptococci is associated with an adhesion to un-mineralized collagen, which might explain the presence of bacteria in the odontoblast processes[25,26].

The findings in the dentin affected by caries differed considerably from what was found in the normal dentin with dentin breakdown and massive staining of bacteria, which was also seen in the SEM analyses. Therefore, the processes in carious dentin must be regarded as totally different from those seen in the dentin below hypomineralized enamel.

The pulp reactions found were mainly limited to the presence of reparative dentin, and in only one case could inflammatory cells be found indicating there had been a response to the bacterial invasion of the dentinal tubules. It is reasonable to believe that MIH teeth that remain for a longer period in the oral cavity could create clinically subjective problems with chronic pulpitis. However, further studies of MIH teeth, exposed for longer periods of time in the oral cavity and with subjective symptoms, would therefore, be necessary.

Conclusions

It may be concluded that oral bacteria may penetrate through hypomineralized enamel into the dentinal tubules and create inflammatory reactions in the pulp, thus possibly contribute to hypersensitivity of teeth with MIH. Teeth, diagnosed with MIH, even with apparently intact surfaces, need special attention in order to avoid pulpal complications.

What this paper adds

- This paper shows that it can be concluded that oral bacteria will penetrate through hypomineralized enamel into the dentinal tubules and create inflammatory reactions in the pulp.
- This paper gives an explanation of the cause of hypersensitivity on teeth diagnosed with MIH.

Why this paper is important to paediatric dentists

- MIH is a severe clinical problem that creates big problems for the patient, but also involves severe difficulties for the dentist to find a proper treatment. The most common problems for the patient are tooth collapse and cavitations, loss of fillings, secondary caries, but most of all, severe hypersensitivity. This hypersensitivity often results in severe discomfort, behaviour management problems, and dental fear.
- The teeth diagnosed with MIH, even with apparently intact surfaces, need special attention by the paediatric dentist, in order to avoid pulpal complications. Understanding the mechanisms of the hypersensitivity, of teeth diagnosed with MIH, is necessary, to uncover the best possible treatment.

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