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Scanning Electron Microscopy of Enamel and Dentin of Teeth with Hypocalcified Amelogenesis Imperfecta

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The histological features of teeth with hypocalcified amelogenesis imperfecta (AI) have been poorly studied, which calls into question the effectiveness of modern adhesive techniques used in the treatment of these noncarious defects.

Aim: The aim of this study was to investigate the morphological features of the enamel and dentin of teeth with AI using scanning electron microscopy (SEM), and compare these features with those of healthy teeth.

Materials and methods: We examined four primary teeth extracted on indication from a 10-year-old girl with hypocalcified amelogenesis imperfecta. The same number of primary teeth extracted from healthy subjects was used as controls. The morphological characteristics of the enamel and dentin are described after investigating the teeth and photographing the specimens with scanning electron microscope.

Results: The SEM photos of the enamel of AI teeth show presence of external defects, incorrectly oriented enamel prisms and enlarged interprism spaces. Thickening of the peritubular dentin and partially obliterated dentinal tubules characterize the dentin of these teeth.

Conclusion: The enamel and dentin of teeth affected by hypocalcified AI differ considerably from normal hard dental structures in their morphological characteristics.

INTRODUCTION

Amelogenesis imperfecta (AI) is a term for a heterogeneous group of genetic disorders that affect the dental enamel in its developmental stage. What subtype AI is - hypoplastic, hypocalcified or hypomaturational enamel - depends on the inheritance pattern and the specific clinical features. The biological potential of the disorder is not limited to defects in the enamel. Local characteristics of AI can include unerupted teeth, open occlusion, pulp calcification, dentin dysplasia, crown and root resorption, taurodontism^{1,2} and others. Little known and insufficiently studied are the specific histological features of hypocalcified amelogenesis imperfecta – these could help shed light on what causes the rapid abrasion of AI teeth, their secondary colouring and poor resistance to decay and problems associated with their adhesive restoration. Given that most teeth with AI lose the defective enamel very soon

leaving the dentin exposed to the oral environment, the structure and mineralisation pattern of dentin are of special interest in this condition.

AIM

The aim of this study was to investigate morphologically the enamel and dentin of teeth with AI using scanning electron microscopy (SEM), and compare their morphological structure with that of healthy teeth.

MATERIALS AND METHODS

The study included four primary teeth extracted on indications from a 10-year girl with amelogenesis imperfecta of the hypocalcified type. The diagnosis of amelogenesis imperfecta was made after clinical examination and studying the family history. For controls we used the same number and kind of primary teeth extracted from healthy individuals

during the period of physiological exfoliation.

PREPARATION OF SPECIMENS FOR SCANNING ELECTRON MICROSCOPY (SEM)

After extraction the teeth were prepared for SEM by fixing them in 4% buffered glutaraldehyde solution (0.075 M, pH 7.3) and then stored for 90 min in a cold buffer solution of sodium cacodylate (0.02 M, pH 7.2, 660 mOsm). The subsequent dehydration was performed in ascending grades of ethanol according to the critical point drier (CPD) method in a desiccator. The dried specimens were gold coated (200-250 nm) by cathode atomization under vacuum.

Scanning electron microscopy was carried out at the Institute of Mineralogy and Crystallography at the Bulgarian Academy of Science using electron microscope (Philips SEM 515, Holland), with accelerating voltage of 25kV in secondary electron

emission mode. For each sample five microphotographs were obtained in the frontal and lateral projections with the same magnification (x2000), and various microphotographs with a different magnification.

RESULTS

CHANGES IN THE ENAMEL

The enamel of the affected teeth was white to yellow-brown and without gloss. The dentin in the coronary part was partially exposed because of the abrasion of the enamel, especially in the area of the occlusal contacts (cutting edges, tubercles), which can be clearly seen on the sections we made of some of the teeth with AI (**Fig. 1**). The abraded occlusal surfaces of AI teeth seen in SEM (**Fig. 2**), the dentino-enamel junction cannot be clearly iden-

Figure 1. Partially abraded incisal surface of a primary canine with AI, section, magnification x40 (microphotograph made by Assoc. Professor P. Zagorchev).

Figure 2. Abraded occlusal surface of a primary first molar with AI, SEM, magnification x2000.

Figure 3. Mosaic-like appearance of the enamel surface of a tooth with AI. Numerous shallow depressions, SEM, magnification x2000.

Figure 4. Highly wavy occlusal surface of a tooth with AI, with numerous small enamel pearls, SEM, magnification x2000.

tified, and the exposed dentin had lost its tubular structure, probably because of secondary obliteration.

At high magnification in SEM, the enamel surface of AI teeth looked rough and had a pronounced mosaic-like appearance (**Fig. 3**). The unabraded sectors of enamel (excluding the occlusal contacts) were wavy, with numerous small protuberances (resembling enamel perls), especially at the base of the fissures (**Fig. 4**).

Unlike the AI tooth enamel, the scans of normal enamel (control) show a relatively smooth and even surface (**Fig. 5**). This is the difference from the enamel of affected teeth (their surface is rough and porous) that can account for the greater bacterial plaque accumulation on the latter (**Fig. 6**).

The enamel affected by AI has poorly organized prism structure. There are many shallow pits along

the entire surface of the enamel. The main feature of these pits is that they become cavity at the basis of the enamel. These cavities are most probably enamel prism-free spaces due to abnormal mineralization. Similar defects, although more shallow, were observed in the control group of healthy teeth, but here they are rarely seen and less pronounced.

At large magnification the enamel surface appears mosaic-like with a lot of shallow pits identical to those reported by other authors (**Fig. 7**). The scans show a vaguely delineated prism structure in the greater part of the enamel with the exception of the outer surface, which is not structured. The enamel prisms are irregularly arranged and their crystals are rough and granulated compared with those in normal enamel (**Fig. 8**). The interprism spaces are larger. In general, the enamel of teeth with AI has

Figure 5. Enamel surface of intact normal tooth. SEM, magnification x2000.

Figure 6. Enamel surface of a tooth with AI. It is uneven, porous and covered with bacterial biofilm. SEM, magnification x2000.

Figure 7. A tooth with AI. The enamel surface is the enamel crystals are coarse and granular, chaotically arranged and with defects that extend to the dentino-enamel junction. SEM, magnification x6000.

Figure 8. Irregularly arranged enamel prisms (sagittal and radial), SEM, magnification x4000.

impaired mineralization, and ultrastructural defects in the prism architectonics.

CHANGES IN THE DENTIN

The dentin's scans of the tooth samples with clinically diagnosed hypocalcified type of AI show presence of dentin different from that in the control group. The dentinal tubules in AI teeth have a narrower lumen and are partially obliterated. The borderline between peritubular and intertubular dentin in many of the dentinal tubules cannot be identified. We found sclerotic depositions in the tubular wall (**Fig. 9**). In the control samples of healthy teeth the

Figure 9. Partially obliterated dentinal tubules with narrower lumen and slightly detectable boundary between peritubular and intertubular dentin. SEM, magnification x3000.

Figure 10. Normal dentinal tubules. SEM, magnification x3000.

peritubular and intertubular dentin can be distinctly differentiated and there is no obliteration of the canal structure (**Fig. 10**).

DISCUSSION

Most studies on AI focus on the morphology of abnormal enamel, without analysing in detail the changes in the underlying dentin.^{3,5,6} In this study we show the differences in the morphology of healthy dental tissue in comparison with that in teeth with AI. The specimens of teeth with hypocalcified AI we studied show a thin layer of mineralized surface layer and an adjoining inner zone of enamel hypocalcification in varying degrees. Some researchers report that such a hypocalcified or porous zone can be found also in primary teeth of healthy individuals.³ In the teeth with AI this area is wider and more clearly marked. These results are similar to that reported by Backman et al.⁴ The SEM obtained images of areas with hypocalcification and mosaic-like enamel show a more irregular type in all specimens with AI in comparison with the healthy controls.⁵ The results of the present study corroborate this finding.

In studying the specimens from the base of the enamel we observed irregular structures near the dentino-enamel junction where the prisms were cut sagittally and radially.

This structure is defined by some researchers as disordered cluster of prisms and greater amount of organic material.^{4,6-8} In the affected teeth we studied we observed structural changes in the morphology of the enamel prisms, as demonstrated in other research.⁷⁻⁹ The major difference from the healthy tooth specimens is the presence of hypocalcified enamel with mosaic strips.

In the dentin area the electron microscope images in our study show the morphological peculiarities of the dentin in AI teeth, focusing on the thickening of the peritubular dentin and partial obliteration of the dentinal tubules. We observed also areas of entirely sclerotic dentin. In most tubules the intertubular dentin and the peritubular dentin are not distinctly demarcated. This morphological structure corresponds to the so-called sclerotic dentin. According to Van Meebek this is morphologically transformed tissue in which the dentin is exposed directly to the oral environment, common in AI (hypocalcified type).¹⁰

Bäckman et al. studied the correlation between clinical phenotype and just the right amount of mineral elements measured with different analytical procedures. The hypermineralisation of dentin is associated with changes in morphological characteristics, for instance the sclerotic dentin that is harder than orthodentin.⁴

The data obtained from SEM on the structural

and morphological characteristics of dentin in AI (hypocalcified type) must be interpreted as a defense mechanism against external stimuli that defective enamel (often missing entirely) cannot respond to.¹¹

These structural and morphological characteristics are also important from a clinical point of view, because application of adhesive bonding systems to this dentin to restore the teeth of patients with AI differs considerably from the bonding to normal dentin. The dentin-adhesive systems will be less effective if they are used for sclerotic dentin in comparison with cases where they are used for normal unaffected dentin.¹² The choice of therapeutic approach in patients with AI is often complex. The SEM analysis shows absence of prismatic structure in the enamel, which puts into question whether conventional adhesive systems and composite materials are appropriate for management of AI. Seow and Amaratunge investigate the effect of acid-etching on extracted teeth with AI of the hypocalcified and hypoplastic variety of the disorder. Their results show that the absence of typical etching is probably due to the changed structure of the prisms in the enamel and suggest a change in the etching time and the concentration of the materials used.¹³ Other authors revise the techniques used for etching of hypoplastic enamel and hypermineralised dentin such as laser Er YAG conditioning and other modifications.¹¹ The choice of a reliable treatment protocol is largely determined by the identification of the type of structural defects. That's why some authors treat the hypocalcified surfaces with NaOCl to improve bonding to composite materials.¹⁴⁻¹⁶ This approach, however, would not be appropriate for highly mineralized hypoplastic enamel without increased concentration of protein.

CONCLUSION

The morphologic assessment of teeth affected by AI (hypocalcified type) made using a scanning electron microscope in the present study important and informative. It showed that the architectonics of enamel and dentin in AI affected teeth are completely different from those of normal hard dental tissues, which in combination with the impaired mineralization is the reason for the rapid abrasion of the enamel, discoloration and hypermineralization of dentin and the high predisposition to caries development in AI.

These structural characteristics suggest a different response of the hard dental tissues in AI to

acid etching, which makes it necessary to develop a special adhesive protocol in the restoration of such defects with composite materials.

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Сканирующая электронная микроскопия эмали и дентина зубов с гипокальцифицированным неполноценным амелогенезом

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Гистологические особенности зубов с гипокальцифицированным неполноценным амелогенезом (AI) были слабо изучены, что ставит под сомнение эффективность применения современных адгезивных техник, используемых при лечении данных некариозных поражений.

Цель: Целью данного исследования было изучение морфологических особенностей эмали и дентина зубов с гипокальцифицированным неполноценным амелогенезом с использованием сканирующей электронной микроскопии (СЭМ), и сравнение этих особенностей со здоровыми зубами.

Материалы и методы: Мы исследовали четыре временных зуба, полученных при удалении зубов по медицинским показаниям у 10-летней девочки с гипокальцифицированным неполноценным амелогенезом. То же самое количество временных зубов, полученных при удалении зубов у здоровых испыты-

емых, использовали в качестве контрольного образца. Морфологические особенности эмали и дентина были описаны после исследования зубов и фотографирования образцов с помощью сканирующего электронного микроскопа.

Результаты: На СЭМ -фотографиях эмали зубов видно наличие внешних поражений, неправильно ориентированных эмалевых призм и увеличенных межпризматических пространств. Дентин таких зубов характеризуется техникой, утолщением перитубулярного дентина и частично стертими дентинными каналами.

Заключение: эмаль и дентин зубов, поражённых гипокальцифицированным неполноценным амелогенезом, значительно отличаются от нормальных твёрдых зубных тканей и их морфологических характеристик.

Ключевые слова: неполноценный амелогенез, дентин, эмаль, морфология, СЭМ