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Aspects of the final phase of enamel formation as evidenced by observations of superficial enamel of human third molars using scanning electron microscopy

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ABSTRACT

Objective: Enamel structure reflects ameloblast function. By studying the structure of the superficial enamel, information about ameloblast function toward the end of the secretory stage may be obtained.
Design: The superficial enamel in midcoronal areas of acid-etched facio-lingual sections from human third molars was studied in the scanning electron microscope (SEM).
Results: A great variation was observed in occurrence of prism-free enamel. Prism-free enamel dominated in 40% (mandibular) and 47% (maxillary) of observed areas and had a mean thickness of about 30 μm. Striations in the prism-free enamel had an interstriae distance of about 3.3–3.8 μm. The angle between prisms and enamel surface was about 60°, between prisms and Retzius lines about 45° and between Retzius lines and enamel surface about 15°. The distances between regularly occurring Retzius lines and between striations in the prism-free enamel both tended to decrease toward the enamel surface. Prisms could change direction as they approached the enamel surface, mostly in cervical direction. Where Retzius lines curved and converged occlusally, prisms tended to deviate in an occlusal direction.
Conclusions: Judged from the incremental lines and occurrence of prism-free enamel, ameloblasts slow down and tend to lose their Tomes' process as they approach the end of secretion. The crystals of prism-free enamel belong to the same system as the interprism crystals of prismatic enamel. A method, based on the disposition of fine incremental lines, is suggested for evaluation of ameloblast dynamics in the last stage of enamel secretion.

1. Introduction

Ameloblasts are versatile cells. They produce and secrete the enamel's organic matrix in which crystals are initiated and initially supported, they allow and provide for the growth of the crystals, they organize the crystals into prisms and interprism, contributing to enamel strength, and, finally, the ameloblasts perform a controlled phasing out of enamel formation, providing the tooth with a functional crown morphology.

The structure of the superficial enamel gives information about ameloblast function in the final stage of enamel formation. Prisms evidence the movement of individual ameloblasts while Retzius lines reflect the incremental growth of enamel as the ameloblast layer as a whole moves in centrifugal direction (Boyde, 1976, 1989; Gustafson, 1959; Osborn, 1973; Risnes 1998). Prism cross-striations and regularly spaced Retzius lines reflect rhythms in ameloblast secretion (Asper, 1916; Bromage, 1991; Dean, 1989; Gysi, 1931; Newman & Poole, 1974; Risnes, 1986, 1998; Schour & Poncher, 1937) and prism-free enamel evidences the loss of Tomes' processes (Gwinnett, 1967; Kodaka, Nakajima, & Kuroiwa, 1989; Newman & Poole, 1974; Risnes, 1998).

The superficial enamel is also of great importance from a clinical point of view, since this is the part that interacts with the oral environment (Arends, 1983). This is where the enamel pellicle and dental plaque is formed (Marsh & Bradshaw, 1995; Rosan & Lamont, 2000), where processes of de- and remineralization occur (Arends & ten Bosch, 1992; ten Cate, 1994; Wen, 1989), where enamel caries is initiated (Arends & Christoffersen, 1986; Cevc, Cevc, Schara, & Skaleric, 1980; Hayashi, 1995; Robinson et al., 2000; Shellis & Hallsworth, 1987; Shellis, 1984b; Silverstone, 1973), where fluoride from local fluoride treatment exerts its functions (Castioni, Baehni, & Gurny, 1998; Clarkson & McLoughlin, 2000; Øgaard, Seppa, & Rølla, 1994; ten Cate 1999; ten Cate & Featherstone, 1991; White & Nancollas, 1990), where tooth abrasion/attrition/erosion of various etiologies occurs (Jagger & Harrison, 1995; Kodaka, Kuroiwa, & Kobori, 1993; Lambrechts, Braem,

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Vuylsteke-Wauters, & Vanherle, 1989; Lazarchik & Filler, 1997; Maas, 1991; Moss, 1998; Pintado, Anderson, DeLong, & Douglas, 1997; West, Hughes, & Addy, 2001) and where acid is applied for resin retention (Galan and Lynch, 1993a, 1993b; Gwinnett, 1988).

Transcription factor regulation of enamel genes seems to be important for the extinction of enamel matrix production, i.e. for determination of enamel thickness (e.g. Babajko, de La Dure-Molla, Jedeon, & Berdal, 2015; Lézot et al., 2008). The aim of the present study was to contribute to our knowledge of the structure of the superficial enamel and to provide a morphological basis for an evaluation of the final phase of the ameloblasts' formative function. This concerns primarily ameloblast movement (direction, rate, and timing) and topography of the ameloblast secretory aspect, both of which may be deduced from the enamel structure.

2. Material and methods

The material and methods employed were the same as described by Li and Risnes (2004). The crowns of human third molars were cleaned thoroughly and cut off from the roots. After dehydration in alcohol and acetone, the tooth crowns were embedded in resin. Each tooth crown was sectioned longitudinally in facio-lingual direction through the top of one of the facial cusps using a sectioning machine with a rotating diamond wheel and an adjustable specimen stage (Risnes, 1981). Two additional cuts were made mesially and distally to the first cut, resulting in two 2–4 mm thick longitudinal crown segments. The medial aspect of each segment was further ground with water on 1200 grit silicon carbide paper (3M® Company). The teeth were cleaned by brushing under running tap water and dried with absorbent paper. The specimens were held by a pair of tweezers, taking care not to touch the enamel, and moved back and forth (about 1 cycle per second) in a Petri dish containing 50 ml 1% nitric acid solution for 15 s. The acid was changed for each 15 specimens etched. The specimens were rinsed under running tap and distilled water and air dried for at least one day. They were then fixed to aluminum stubs with cyanoacrylate glue, the unground mesial or distal aspects facing the stub, sputter-coated with about 30 nm gold-palladium and observed in a scanning electron microscope (Philips XL30 ESEM) operated at 12–15 kV. The superficial enamel was observed in standard locations on the facial and lingual aspects, midway between the cusp tip and the cemento-enamel junction. Only areas with intact enamel were included in the study. All sections were examined at a tilt angle of 0°. Linear and angular measurements were performed manually on micrographs with ×500 magnification. The measurements were performed one time by one investigator. Standard statistical analyses were performed (mean, standard deviation, and significance according to Student's *t*-tests of unpaired/independent samples).

3. Results

3.1. Occurrence of prism-free enamel

In the superficial enamel prisms might or might not reach the enamel surface. Where the prisms did not reach the surface, a zone of prism-free enamel was present. However, the transition from enamel with prisms to enamel without prisms was not always distinct, the superficial enamel often exhibited a mixture of prisms and prism-free enamel. Based on a subjective evaluation, the enamel in the areas observed was assigned to one of the following two types:

I. Prisms predominate, but scattered areas of prism-free enamel or a very thin layer of prism-free enamel may be present (Fig. 1)

II. Prism-free enamel predominates, but scattered prisms may be present (Fig. 2)

Type I occurred somewhat more frequently than Type II both in maxillary and mandibular third molars, in 53% and 60% of the observed areas, respectively (Table 1). Prism identity could become obscured without being totally lost (Fig. 1b) and such areas were consequently assigned to Type I. Termination of prisms could occur in a scattered fashion with zones or patches of prism-free enamel extending irregularly into the enamel (Figs. 1b,d, 2a,c–d,f). Groups of prisms could terminate at the same level, creating a prism-free enamel of uniform thickness (Fig. 2b). There was a tendency for prisms to terminate at distinct Retzius lines (Figs. 1d, 2a,d–e). However, scattered prisms or groups of prisms could continue beyond the Retzius line (Figs. 1d, 2a,d–e). The thickness of the prism-free layer in Type II areas was 28 ± 13 µm in maxillary molars and 31 ± 15 µm in mandibular molars (Table 1).

3.2. Prism cross-striations and striations in prism-free enamel

Prism-cross striations were variably expressed and exhibited an interstriae distance between 3 and 6 µm (Figs. 1a,d,f, 2a–b,d,f). The prism-free enamel often exhibited a striation oriented parallel with the Retzius lines and being closely related to the prism cross-striation periodicity (Figs. 1d–f, 2a–b,d,f). Striations in interprism domains showed an orientation similar to the striations in the prism-free enamel (Fig. 2d). There was a tendency for the distance between the striations in the prism-free enamel to decrease toward the enamel surface (Fig. 2 d,f), from 3.8 to 3.6 µm in maxillary molars and from 3.5 to 3.3 µm in mandibular molars (Table 1). However, these differences were not significant (Student's *t*-test, $p \gg > 0.05$). In some areas the striations were very compressed, with a periodicity below 2 µm (Fig. 2f). A tendency for convergence of striations was also observed (Fig. 2d).

3.3. Prism course

In areas with unambiguous directions of prisms and Retzius line(s), the angles between prisms and enamel surface, between prisms and Retzius lines, and between Retzius lines and enamel surface were measured (Figs. 1a–c,e, 2a–b). For maxillary and mandibular third molars, respectively, the angles were 61 ± 7° and 60 ± 6° for prisms/surface, 46 ± 7° and 46 ± 6° for prisms/Retzius lines and 15 ± 4° and 14 ± 4° for Retzius lines/surface (Table 1).

In about 15% of the areas observed prisms changed direction as they approached the enamel surface, mostly in a cervical direction (Figs. 1d,f, 2c). However, in some areas prisms could deviate in an occlusal direction (Fig. 2e–f). In such areas there was a tendency for the incremental line pattern to be compressed.

3.4. Retzius lines

Retzius lines were often a prominent feature of third molar superficial enamel (Figs. 1b,e–f, 2a,d–e) although in some areas they were less conspicuous or even absent (Figs. 1a,c–d, 2b–c,f). The Retzius lines were often spaced at regular intervals, but there was a tendency for the inter-Retzius line distance to decrease in centrifugal direction, from 49 µm between 3rd and 4th to 36 µm between 1st and 2nd in maxillary teeth and correspondingly from 45 µm to 36 µm in mandibular teeth, measured along the prisms (Table 1) (Figs. 1b,e, 2a,e). Using Student's *t*-test the distances between Retzius lines were found to be significantly different or probably significantly different from each other (R1–R2 versus R2–R3: $p \ll 0.01$ for maxillary molars, $p \ll 0.05$ for mandibular molars; R1–R2 versus R3–R4: $p \ll 0.01$ for both molars), except for the distances R2–R3 versus R3–R4, which were not significantly different ($p \gg > 0.05$).

Retzius lines may end up in more or less distinct perikyma grooves on the enamel surface (Figs. 1b,e–f, 2a,d–f).

4. Discussion

The variation in superficial enamel structure, especially concerning loss of prism identity and expression of prism-free enamel, was striking