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# Enamel Structure and Forensic Use

[1]C. Stavrianos, [2]C. Papadopoulos, [1]L. Vasiliadis, [3]P. Dagkalis, [1]I. Stavrianou and [1]N. Petalotis
[1]Department of Endodontology (Forensic Odontology),
School of Dentistry, Aristotle University, Thessaloniki, Greece
[2]Department of Forensic Sciences (Forensic Odontology), University of Glamorgan, Wales, UK
[3]Department of Preventive Dentistry Periodontology and Implant Biology,
School of Dentistry, Aristotle University, Thessaloniki, Greece

**Abstract:** Tooth enamel is the hardest and most highly mineralized substance of the body and with dentin, cementum and dental pulp is one of the four major tissues which make up the tooth in vertebrates. The unique microstructure of enamel resides fossilized tracks of its growth process. These tracks represent the incremental growth of enamel. Forensic odontology is a vital and integral part of forensic science. Essential data could be derived by the study of the enamel structure and contribute in dental identification, age estimation procedures and archaeology, anthropology and forensic researches. Further advances in image analysis and computer technology would enhance the knowledge and improve the accuracy of methods used in Forensic odontology field.

**Key words:** Forensic odontology, enamel, neonatal line, dental identification, age estimation, vertebrate

## INTRODUCTION

Mature enamel is a non-vital, non-cellular highly mineralized dental tissue which forms the outer layer of the tooth. It is heavily mineralised and is being formed by regularly packed cells called ameloblasts. The enamel is in general characterized by the discontinuities that were established during matrix secretion by the ameloblasts. The unique microstructure of the enamel resides fossilized tracks of its growth process. These tracks represent the incremental growth of enamel (Fig. 1), i.e., retzius lines, perikymata and prism cross-striations and are considered to be micro structural growth markers (Hillson, 1996; Risnes, 1998; Katzenberg *et al.*, 2005; Nanci, 2008; Mishra *et al.*, 2008; Xie *et al.*, 2009).

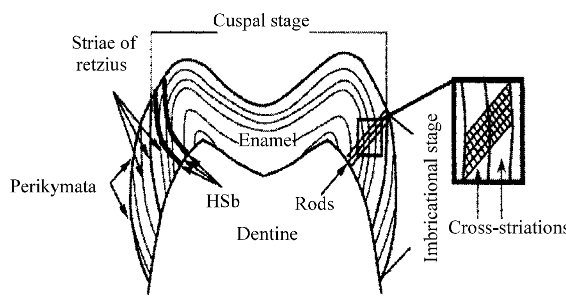


Fig. 1: Schematic section of a tooth showing incremental lines in enamel (Rozzi, 1998)

## STRUCTURE OF THE ENAMEL

Enamel contains on average 95% inorganic matter, 4% water and 1% organic matter by weight or 87% inorganic, 11% water and 2% organic component by volume and consists mainly of hydroxyapatite crystals.

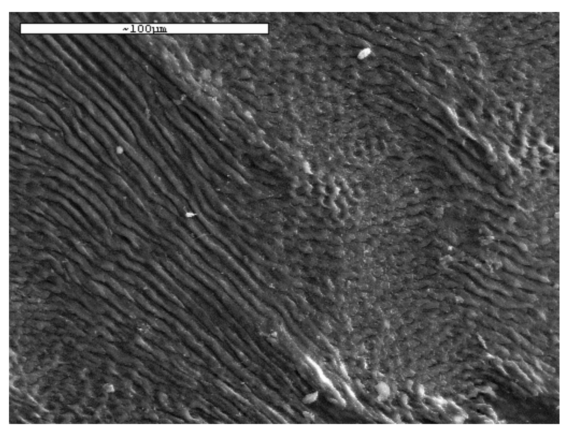


Fig. 2: Surface of mature enamel showing evidence of the prism structure

**Prisms:** The fundamental units of enamel structure are bundles of crystallites known as prisms (or rods, Fig. 2). The structural complexity of dental enamel is related to the way in which hydroxyapatite crystals are arranged. Each

**Corresponding Author:** Stavrianos Christos, Department of Endodontology (Forensic Odontology), School of Dentistry, Aristotle University, Thessaloniki, Greece

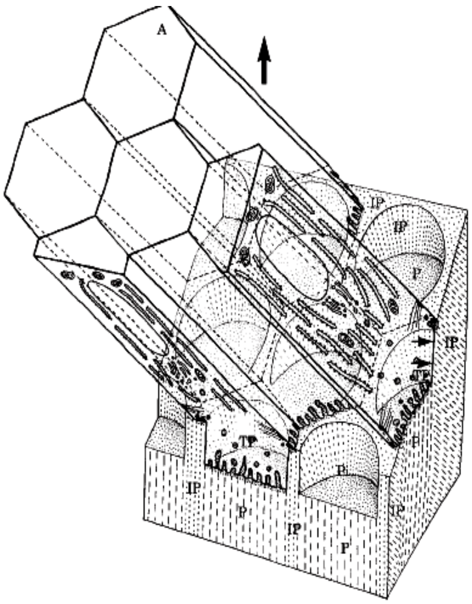


Fig. 3: Schematic representation of developing enamel. Crystal orientation is indicated by hatching/stippling. P = Prism, IP = Interprism (Risnes, 1998)

ameloblast is responsible for a prism in mature enamel. Initially, the enamel has no prisms as the flat distal ends of the ameloblasts secrete it. It is after the development of Tomes' processes that the prism (rods) and interprism (interrod) patterns appear on crystal orientation (Fig. 3). Prism width, 5 μm at the dentin-enamel junction, 10 μm at the outer enamel layers (Risnes, 1998; Tziafas, 1999).

The only difference between them is the orientation of the crystals. In prisms, the crystals are generally oriented parallel with the prism axis whilst in interprisms they are oriented perpendicular to the incremental lines. However both within prisms and interprism, there is some variation in crystal orientation which may reflect a variation in the topography of the secretory surfaces of ameloblasts.

Three main types of prism patterns have been described. Human enamel belongs to pattern three in which the prisms have a keyhole pattern (Fig. 4a-c and 5) in transverse section (Hillson, 1996; Risnes, 1998; Rozzi, 1998; Radlanski *et al*., 2001; Mishra *et al*., 2008; Nanci, 2008; Antoine *et al*., 2009; He and Swain, 2009).

**Prism decussation:** The paths of enamel prisms do not run straight throughout the thickness of enamel and groups of prisms undulate from side to side in a sinusoidal or helicoidal fashion. This phenomenon is described as prism decussation by Antoine *et al*. (2009).

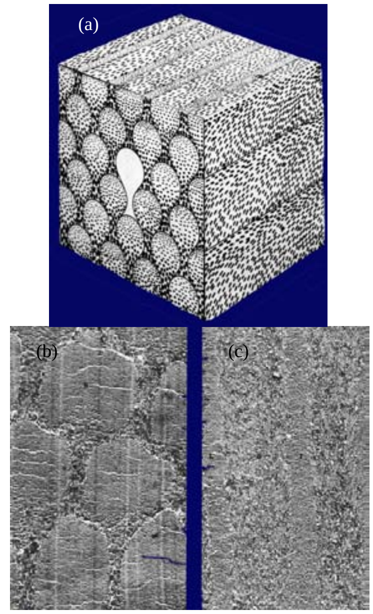


Fig. 4: Schematic illustration of enamel keyhole-shape rod structure; a) TEM microphotographs of the enamel prisms. Surface of key hole appearance of the cube; b) Upper surface of the cube and c) original magnification, 5000 X

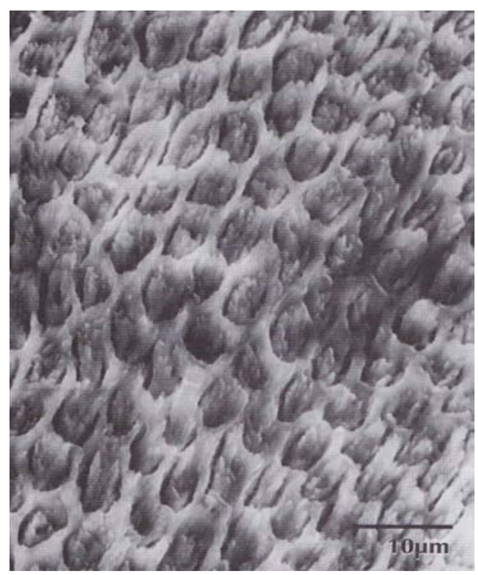


Fig. 5: SEM photograph of transverse sectioned enamel rods at the stage of active amelogenesis (1500 X, Tziafas, 1999)

**Prism cross-striations:** The prism cross-striations are corresponding periodic features visible in the light microscope as slight striations perpendicular to the axis of the prism at regular intervals of approximately 3-4 μm (range 2-8 μm). They appear as successive dark and light bands along prisms in a scanning electron microscope. The prism cross-striations are the result of a circadian rhythm in the metabolic activity of ameloblasts. There is circumstantial and experimental evidence that supports the theory that prism cross-striations represent a daily rhythm in enamel growth. Cross striations result from variations in the degree of mineralisation of enamel and the rate of secretion (Hillson, 1992; Risnes, 1998; Rozzi, 1998; Katzenberg *et al*., 2005; Mishra *et al*., 2008; Antoine *et al*., 2009). Risnes (1986) calculated the mean rate of enamel apposition to be about 3.5 μm that fits with the cross-striation periodicity most often met with in the literature (3-4 μm).

**Retzius lines:** The retzius lines or striae of retzius are growth lines of the enamel that represent growth planes with a three-dimensional extent and they are oriented perpendicular to the direction of appositional growth. They reflect the variations in structure and mineralization. In the light microscope, a large number of retzius lines could be observed in ground section of permanent teeth. They represent a regular growth rhythm. Assuming that the prism cross-striations represent a daily rhythm in enamel production, the periodicity of the rhythmic retzius lines can be determined by counting the number of cross-striations between two lines. For human enamel, a mean of 7-8 prism segments between two lines with a range of 6-10 has been found indicating a close to weekly periodicity of the rhythmic retzius lines. The striae arrangement divides the crown of the tooth in two stages. The cuspal (appositional) stage where the striae have not reached the enamel surface and involves successive layers of appositional enamel. The imbricational stage where the striae reaching the enamel surface to produce the perikymata (Hillson, 1992; Risnes, 1998; Rozzi, 1998; Mishra *et al*., 2008).

**Perikymata or imbricational lines:** Perikymata are furrows or grooves that are created where the retzius lines reach the enamel surface (Fig. 6). In human deciduous teeth, perikymata are much more scarce due to the scarcity of retzius lines, especially in the prenatal enamel (Hillson, 1992; Risnes, 1998; Mishra *et al*., 2008).

**Neonatal line:** Generally in all deciduous teeth, a distinctive line may be seen in the enamel corresponding to the time of birth, the neonatal line (Fig. 7). It is a prominent and exaggerated incremental line that sometimes appears in the 1st permanent molars as well. It has been recognized as a structural response to disturbances in the environment and nutrition at the event of birth. The appearance of the neonatal line in a light microscope and microradiographs is an optical phenomenon due to alterations in height and degree of mineralization of the enamel prisms. A normal neonatal line has a width of 12 μm but infants who experienced long deliveries have up to a 24 μm wide. The presence of the neonatal line makes possible the distinction of prenatal and postnatal enamel (Teivens *et al*., 1996; Mishra *et al*., 2008; Sabel *et al*., 2008; Antoine *et al*., 2009).

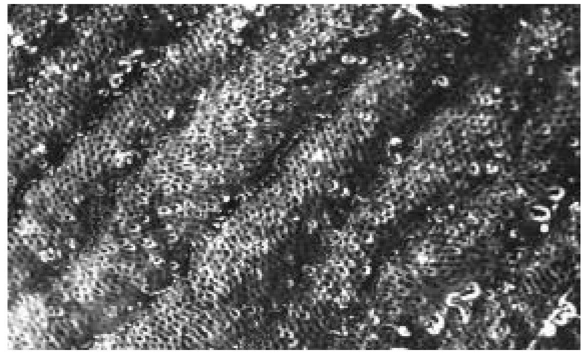

Fig. 6: Perikymata or imbricational lines (1000X)

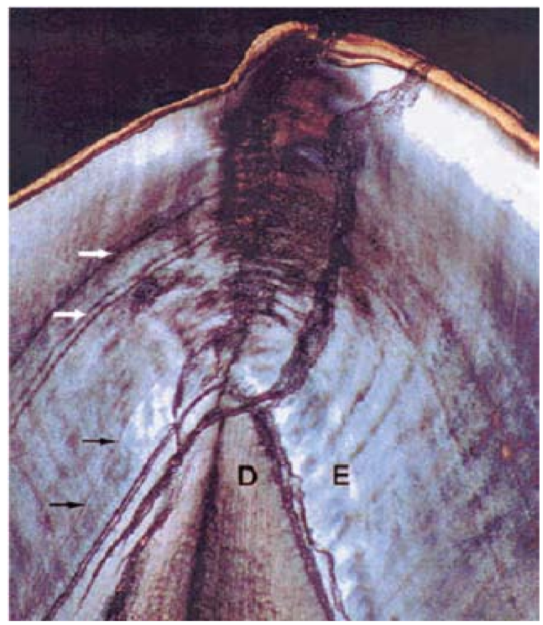


Fig. 7: Transmitted light microscope image of the neonatal line (black arrows) which can clearly be seen in the enamel (E). Examples of accentuated striae (white arrows) can also be observed cusp (Antoine *et al*., 2009)

## DIFFERENCES BETWEEN DECIDUOUS AND PERMANENT DENTITION

In general, there are no principle differences in the enamel structure between the deciduous and the

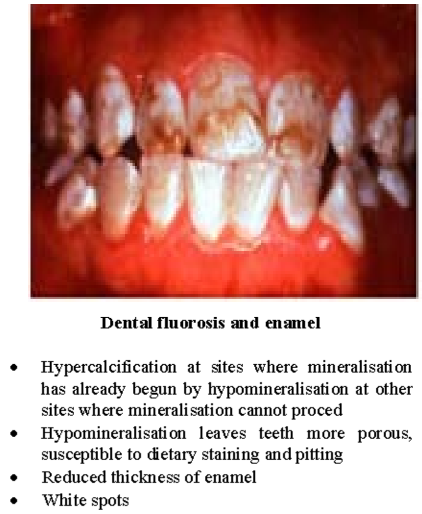


Fig. 8: Forensic odontology application of dental fluorosis. Characteristic of hard dental tissues. Could help in identification in mass disasters and crime investigations. Indication of origin and habits

permanent teeth. Radlanski *et al*. (2001) showed that the outline and the behavior of the rods in deciduous and permanent enamel were the same except the fact that the rods were smaller in deciduous enamel. Risnes (1998) and Whittaker (1982) reported that the superficial enamel might be prism free. Deciduous enamel is less thinner than the permanent and retains lower mineral content (Wilson and Beynon, 1989; Shellis, 1984; Hillson, 1996).

Neonatal line appears in all deciduous teeth and occasionally in permanent molars (Mishra *et al*., 2008; Antoine *et al*., 2009). Additionally, Mishra *et al.* (2008) report that the striae in primary enamel are almost parallel with the enamel surface because of the relatively extensive developmental surfaces of the deciduous teeth.

## FORENSIC APPLICATIONS OF ENAMEL EXAMINATION

Forensic odontology is a vital and integral part of forensic science. Essential data could be derived by the study of the enamel structure.

**Dental identification:** Forensic odontology plays a crucial role in personal identification of unidentified bodies in crime scene investigations and in mass disasters. Unique individual characteristics of the dentition could assist the identification procedure. The study of the enamel reveals information that allows the Forensic odontologist to build up a picture of the deceased's habits and ethnicity. This method helps in synthesis of the dental profile of an

Fig. 9: Amelogenesis Imperfecta (AI). Stainless steel crows are indicated in the primary and permanent dentitions. In hypocalcified and hypomaturation AI types where the enamel is of insufficient strength to retain bonded or intracoronal restorations, full coverage restorations should be placed

individual. Information for the occupation, dietary habits, dental and some systematic diseases could be obtained by a careful examination of the enamel tissue. Erosions could be associated to many factors such as alcohol and substance abuse, working in industrial environment with acid use, consumption of carbonated drinks and disorders like anorexia nervosa.

Stains may suggest smoking, tetracycline use or dental fluorosis (Fig. 8). The latter could be an indication about the ethnic origin of an individual or at least where the childhood was spent. The notching of incisors enamel gives indication for occupation or habits. Also, amelogenesis imperfecta (Fig. 9), represents a group of hereditary enamel defects that are apparently heterogeneous in their basic biochemical defects and which present diverse clinical characteristics. This could also help in identification in mass disasters and crime investigations.

The method of dental profiling of a deceased person or human remains is used in cases where other methods of identification are not sufficient to establish the identity (Hardy, 2007; Shekar and Reddy, 2009; Xie *et al*., 2009; Ohtani *et al*., 2009).

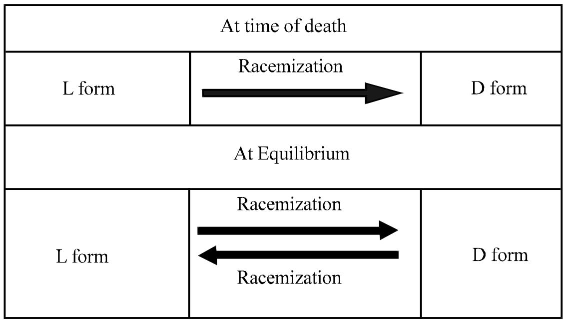


Fig. 10: Racemization. Chiral molecules have two forms (at each point of asymmetry) which differ in their optical characteristics: the levorotatory form (the L form) will rotate the plane of polarization of a beam of light to the left, while the dextrorotatory form (the D form) will rotate the plane of polarization of a beam of light to the right. The two forms are non- superimposable. During life all amino acids are in the L form. At death, there is a conversion to the D form. Measuring the ratio of L:D can give an indication of age at time of death

**Age estimation:** The incremental growth tracks represent an internal record of time which may serve as a valuable tool in determining the developmental and chronological history of a crown. Dependency of enamel microstructures (cross striations, striae of retzius and the perikymata) underlies all dental histological techniques that estimate age or development from enamel. Antoine *et al.* (2009) confirmed that the cross-striations represent a circadian rhythm in humans and that it is possible to make reliable counts of them using routine sections and light microscopy. In their study, after the position of the neonatal line in the enamel was taken into account, the total cross-striation counts were highly consistent with those expected from the known ages (Risnes, 1998; FitzGerald, 1998; Antoine *et al*., 2009).

The method of amino acid racemization in enamel could be used for determination of the age to an accuracy +/- 4 years in forensic work. Aspartic acid is the most commonly used amino acid for age estimation purposes due to its fast racemization at body temperature. Amino acids they exist in two forms, the L and D. Initially when the proteins are formed, all the amino acids are in the L form but during the life of the protein they gradually convert to a mix of L and D forms. This procedure is called Racemization (Fig. 10). This reaction is strongly temperature dependent so, it is important to know the conditions of the burial. Human enamel shows a steady increase in racemization with age which could be used to estimate age at death. Presence of dental decay has small defect on the racemization process (Griffin *et al*., 2008; Shekar and Reddy, 2009). It is very important in forensic cases the discrimination between stillborn infants and those dying later. The neonatal line is described as a band between pre- and postnatal enamel and it is visible provided that the infant survives for at least 7-10 days. It provides an accurate standard in order to distinguish perinatal death and later survival. The amount of enamel formed after birth can be measured to evaluate the duration of postnatal survival. It is has been suggested that after the identification of the neonatal line in the enamel, it is possible to estimate age from prism cross striation counts (Hillson, 1996; Smith and Avishai, 2005).

**Archaeology and anthropology research:** The cross-striation periodicity linked to a diurnal rhythm in enamel formation and extended to retzius line and perikymata periodicity could be used in mapping of the rate and chronology of dental development in hominids. The histological examination of the incremental structures on the enamel surface have been applied in archaeological researches for estimating the age at which growth disruptions caused developmental defects occurred. Crown development chronologies based upon counts of retzius striae and perikymata could provide estimation of age at the time of death in archaeological and fossil hominid specimens. The advantage offered by estimating age and development using the examination of enamel is very important. Firstly, they overcome the problem of the precise and accurate correlation between biological age and chronological age. Secondly, estimating age at death and development of extinct hominoids using standards derived from the fossils themselves, rather than relying on studies of modern human or ape analogues, offers significant benefits in palaeoanthropology (Hillson, 1992; Risnes, 1998; FitzGerald, 1998).

**Research for forensic purposes:** Rythen *et al.* (2008) concluded that enamel disturbances such as hypomineralized areas and the incremental lines in the post-natal enamel are more frequent in primary teeth from preterm children with very low gestational age.

Teivens *et al.* (1996) studied incremental lines of the enamel from infants deceased by Sudden Infant Death Syndrome (SIDS). Previously healthy infants appear to have normal mineralisation pattern with only occasional accentuated incremental lines. On the other hand, children deceased of chronic disease or who had a history of acute serious disease, exhibited enamel with one or more accentuated incremental lines. They concluded that the examination of changes in the enamel structure could help in the evaluation of causes of death in children suspected to have died from SIDS. Also, since inorganic traces of elements taken during enamel mineralization remains in the enamel for ever. This may be useful in identification through medical history and place of birth and growth of the individual.

## CONCLUSION

Further, advances in image analysis and computer technology would help the development of more sophisticated and accurate observation techniques of the mature enamel microstructure. Thus, this would enhance the knowledge and improve the accuracy of methods used in Forensic odontology field.

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