

Scanning Electron Microscope Corroboration of Amelographics – A New Tool in Forensic Odontology

Abstract

Background: Human teeth resist decomposition to the maximum and has immense potential to serve as hard-tissue counterpart to dermatoglyphics in forensic odontology. Amelographics is the science of recording and analyzing the tooth print. **Aims and Objectives:** To assess the scope of viability, reproducibility, and identification of enamel prints (akin to fingerprints) and their patterns as a tool for identification. To establish that expression of enamel prints is a direct result of the enamel rod configuration on the surface of the crown as detected by scanning electron microscope (SEM). **Materials and Methods:** The teeth samples ($n = 10$) were first analyzed through (SEM) and the image of the arrangement of rods on the surface was captured. Enamel prints were registered in a standard procedure by virtue of ink transfer on a cellophane tape from etched tooth enamel surface of the same samples. These prints and SEM images were subjected to Rapid Sizer® image editing software to obtain a pattern (sketched outline image software). Patterns were identified manually. **Results:** Reproducibility, specificity, and feasibility of the above procedure were determined. There appeared to be a high rate of reproducibility (98%–100%) and specificity (100%). The paraphernalia required as well as the technique entrenched were feasible. Furthermore, the SEM analysis established the viability and reliability. **Conclusion:** Amelographics is a sensitive and reproducible scientific tool that can be utilized for the management, examination, and evaluation of dental evidence for identification at crime scene and disaster sites. Its importance vis-a-vis fingerprints cannot be understated, especially in view of the seeming indestructibility of the enamel.

Keywords: Amelographics, enamel rod configurations, scanning electron microscope

Introduction

Advances in forensics have raised a need for the development of more sustainable tools and techniques in victim or convict identification in disasters/crime scenes. Human enamel being tough resists degradation more than any other tissue in the body; therefore, it has immense potential to serve as an identification tool (akin to dermatoglyphics) in forensic odontology.^[1] Amelographics is the science of recording and analyzing the tooth print, an external manifestation of enamel rod ends, with a distinct pattern.^[2]

Although DNA analysis serves as a gold standard in identification, it may not be feasible in severely mutilated cases.^[3] Dependency on dermatoglyphics in soft tissues which are easily friable, limit its use.^[4] Enamel is precisely delineated natural composite and acts as an outer cover of teeth structure. Such microscopic

bundles of crystallites known as prisms are fundamental units of enamel.^[1] Such a design makes it hardest, stiffest, and one of the most durable load-bearing tissues of the human body. Teeth contain a microscopic record of their growth which is very emblematic of every tooth in a person. The structural uniqueness can be attributed to the arrangement of the crystals. Such a variation in crystal orientation is due to the varied topography of the secretory surfaces of ameloblasts, a result of growth and environmental influences. Prisms lace and manifest as enamel surface carvings.^[5] Scanning electron microscope (SEM) investigations show that the undulation of prisms occur from side to side in a sinusoidal and helicoidal fashion and is described as prism decussation. Amelographics is the science of recording and analyzing the teeth prints, manifested by the arrangement of prisms.^[6]

Development of such a concept would augment existing techniques in forensic

Kanika Singroha,
Abhishek Banerjee¹,
V V Kamath²,
J Pramod³,
Saha Alangkar⁴,
E Elampovai⁵

Private Practitioner, JDC, Jodhpur, ¹Department of Oral Pathology and Microbiology, Awadh Dental College and Hospital, ²Department of Oral Pathology, Awadh Dental College, Jamshedpur, Jharkhand, ³Department of Oral Pathology, Dr. Syamala Reddy Dental College and Research Centre, ⁴Department of Oral Pathology, NSVK Dental College Hospital, Bengaluru, ⁵Department of Oral Surgery, Bardhaman Dental College and Hospital, Bardhaman, West Bengal, India

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Address for correspondence:

Dr. Abhishek Banerjee,
5/17 Ambagan, Benachity,
Durgapur - 713 213,
West Bengal, India.
E-mail: abhishek.banerjee376@gmail.com

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science for better identification of the individuals involved in a crime, identification of mass disaster victims as teeth can survive as evidence in very hostile conditions as well.

The purpose of the study is to highlight that the software developed for recording fingerprint patterns cannot be applied to record enamel prints too.

Materials and Methods

The sample size comprised crowns of ten extracted central incisors collected randomly from both male and female patients. The samples included were in the mean age range of 40–80 years. The samples were collected from the patients advised for extraction as they were diagnosed with chronic generalized periodontitis and had grade III mobile central incisors. Care was taken not to include carious teeth. However, patients with fracture line limiting to enamel only were included in the sample. The study was carried out on the intact labial surface of crowns of maxillary permanent central incisors sectioned at cement enamel junction. It was then etched with 37% phosphoric acid for 45 s and rinsed with distilled water. The etched surface was then dried with 20% acetone carried on a cotton bud, applied for 10 s.^[7] Crowns of the central incisors were coated with gold for SEM analysis [Figure 1]. The SEM topographical analysis was carried out at scale bar 50 μ and 100 μ and microphotographs were taken. (The SEM study was carried out in the Department of Material Sciences at Indian Institute of Sciences, Bangalore).

Amelogyphics (enamel prints)

The labial surface of the central incisor was cleansed under running water to wash the gold layer away and air-dried. Ink was impregnated on the marked area using preinked strips. Uniform pressure was applied to the ink strip using cotton buds for 30 s. The print was lifted placing a cellophane tape, subjected to uniform pressure with cotton



Figure 1: Gold coating of the tooth surface for the scanning electron microscope analysis

buds for 15 s. The print on cellophane tape was mounted on the slide. Microphotographs were taken at $\times 10$ of an Olympus 20i microscope.

Pattern sketching

The image was then run through Rapid Sizer® image editing software (Rapid resizer, Patrick Roberts, Collingwood, Ontario, Canada) to obtain a pattern (sketched outline image software). Rapid Sizer® is a pattern sketching software identified from freeware on the net. Permission was obtained from the developer before usage. This software outlined the prism images and created a sketch similar to that done by Verifinger® for fingerprints. The outlined patterns greatly enhanced the groupings of the enamel prints and its identification.

Results

Analysis of amelogyphics

The enamel prints resembled geometry of enamel prisms in SEM [Figure 2a and b] and were manually grouped into three categories: (1) long prism patterns [Figure 3a-c]; (2) short prism patterns [Figure 4a-c]; (3) combination of the above (long prism short prism [LPSP] [Figure 5a-c]).

Long prism pattern was seen in five of the patients, Three exhibited short prism pattern, and two had a combination of LPSP pattern. On comparison of the patterns deduced using Rapid Sizer® image editing software with patterns on SEM images high rate of reproducibility (98%–100%) and specificity (100%) were obtained.

Discussion

Enamel has the potential to be preserved unaltered for many years. Permanent paleontological record of enamel is preserved as it is not remodeled during lifetime. During the formative stage as the ameloblasts develop a unique pattern rendered to enamel which is specific to every species. Any alteration during the time of development is evinced as a structural defect or disturbance later on.^[8,9] Therefore, enamel prints muffled in the enamel must

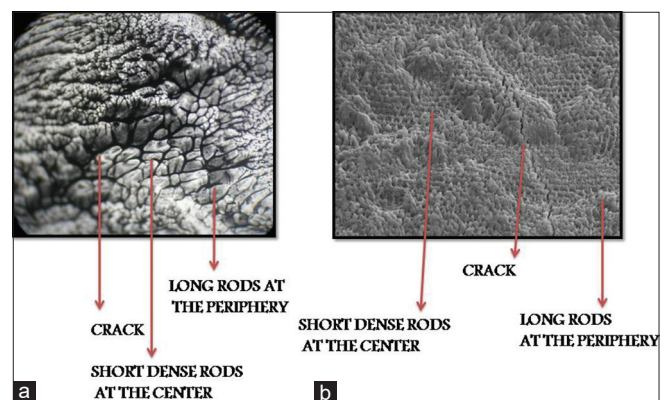


Figure 2: The enamel prints resembled geometry of enamel prisms in scanning electron microscope. (a) Microphotograph of enamel print, (b) scanning electron microscope picture of the enamel prisms

serve as hard tissue alternative to fingerprint patterns as in dermatoglyphics for forensic use.^[10]

In the present study, it was found that it is possible to register prints from enamel similar to dermatoglyphics. The unique pattern is established for every individual as the process of amelogenesis is genetically regulated. Print patterns in the present study both on the cellophane tape and corresponding SEM patterns were very unique and specific to a particular individual. The image editing software Rapid Sizer[®] outlined the prism images and created a sketch similar to that done by Verifinger for fingerprints. The outlined patterns were grouped into three

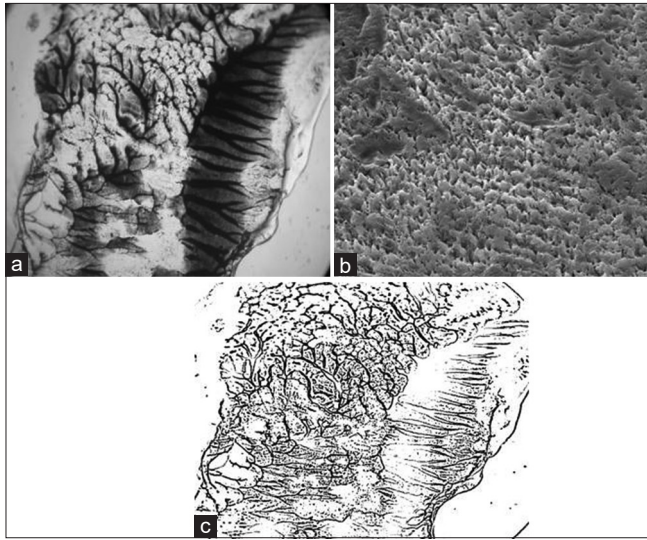


Figure 3: (a) Microphotograph of enamel print long prism patterns. (b) Scanning electron microscope picture at 50 μ depth long prism patterns. (c) Pattern showing long prism patterns

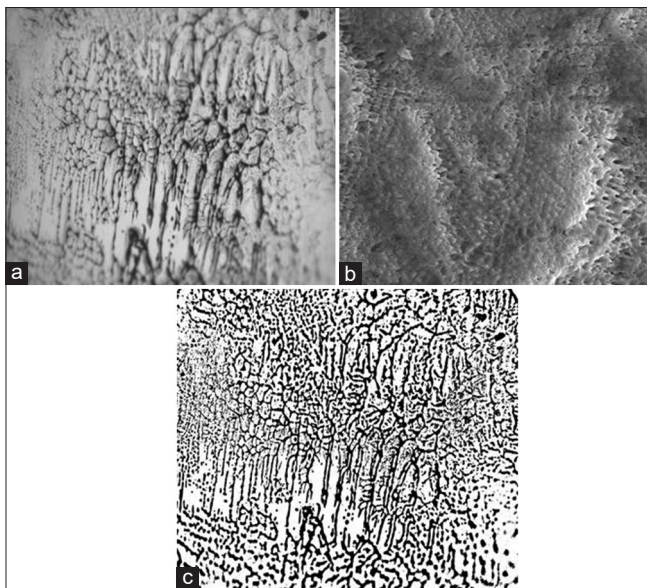


Figure 5: (a) Microphotograph of enamel print long prism short prism. (b) Scanning electron microscope picture at 50 μ depth long prism short prism. (c) Pattern showing long prism short prism patterns long prism short prism

groups: Group I: Long prism pattern, Group II: Short prism pattern, and Group III: Combination pattern. The grouping was done based on the predominant rod structure, that is, if a sample showed >50% long prisms, it was included under long prism pattern and if the short rods comprised >50%, it was called as short prism pattern. The sample with equal components of both the patterns was called as combination pattern. This attempt of manual pattern segregation was similar to that of Boyde.^[11]

Boyde (1964) investigated the enamel surface at various stages of development under SEM for the first time. He integrated observation of developing enamel with enamel prism packing patterns and described them as Pattern 1, 2, and 3 [Figure 6]. The formation of each pattern was attributed to a characteristic number of ameloblasts: Pattern 1 (formed by one cell), Pattern 2 (by two cells), and Pattern 3 (by three cells and a small contribution by

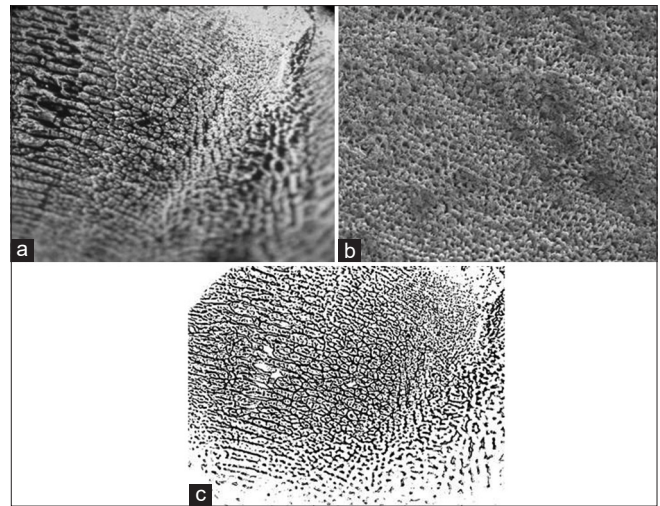


Figure 4: (a) Microphotograph of enamel print short prism patterns. (b) Scanning electron microscope picture at 50 μ depth short prism patterns. (c) Pattern showing short prism pattern

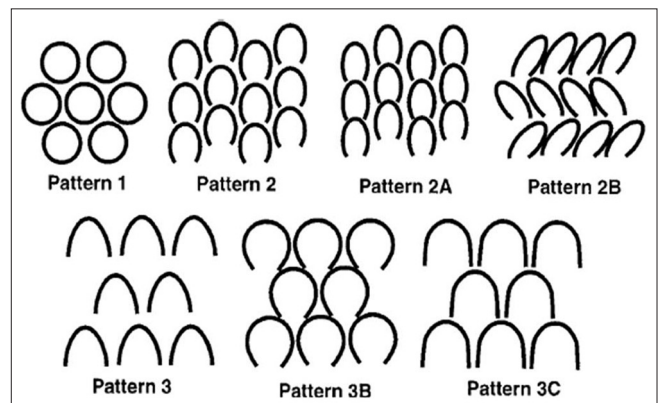


Figure 6: Boyde's prism patterns. Pattern 1 prisms are usually small (3–5 μm) with complete circular boundaries. Pattern 2 prisms are small 2–4 μm diameter and are arranged in longitudinal columns from the apex to the cervix. Pattern 2 variants (Patterns 2, 2A and B) Pattern 3 prisms are larger 5–8 μm in diameter, the variants (Pattern 3, 3B and C) differ in the shape of the prism sheath

the fourth ameloblast). Replicas of acid-etched enamel were studied (these replicas preserved details of a prismatic surface).^[11] Boyde's prism patterns. Pattern 1 prisms are usually small (3–5 μm) and have complete roughly circular boundaries. Boyde's Pattern 2 and Pattern 3 established two major classes of prisms with incomplete prism boundaries and arc-shaped prism sheaths. In both of these prism patterns, the open side of the prism is toward the cervix (root-crown junction) of the tooth. Pattern 2 prisms are small 2–4 μm in diameter and are arranged in longitudinal columns from the apex to the cervix. Pattern 2 variants (Patterns 2, 2a, and 2b) differ in the amount and distribution of interprismatic enamel and angles between prisms in adjacent rows. Pattern 3 prisms are larger 5–8 μm in diameter and packed in horizontally offset rows, like Pattern 1 prisms. The variants (Pattern 3, 3b, and 3c) differ in the shape of the prism sheath and the amount and distribution of interprismatic enamel.

Ronnholm (1962) noted that in Patterns 1 and 2, interprismatic material lies between true prisms, whereas in Pattern 3, prism heads (circular regions) are separated by prism tails, there is no true interprismatic enamel.^[12]

Although Boyde's scheme has been widely used in primate enamel studies, it has been suggested that prism patterns in many primate species are so variable that it may be more practical to distinguish only between "closed" (Pattern 1) and "open" (Pattern 2 and 3) prisms.^[8]

The microphotographs of the prints correspond to the above patterns of enamel as seen under the electron microscope and also simulated the pattern descriptions attempted in the previous studies, thereby establishing the authenticity of the technique used.

The enamel prints for teeth registered in the present study showed a similar combination of prism patterns which form the basis for identification. We have corroborated the expression of the pattern by SEM. The significant feature of this technique is the print pattern corresponds to the prism pattern of the enamel rods.

Radlanski *et al.* demonstrated that it is possible to follow a prism from the enamel dentin junction (EDJ) to the surface using SEM serial sections and confocal laser scanning microscopy imaging. Thereby opening a scope for digital storage.^[13]

The method employed is feasible and reproducible. When the master set was compared with random copies of the sets, a 100% recall and match of all the prints were obtained for both SEM pattern and corresponding cellophane print.

The present technique appears to be compatible for digital assessment as well. Photographs of the prints were digitized into image files at standard magnification and resolution using Adobe Photoshop version 12.0 (adobe systems, Adobe World Headquarters, San Jose,

California, U.S.). Patterns of these images were obtained with the help of a pattern producing software RapidSizer which created wavy patterns of the images. The patterns contained all the variations presented in the ink–prints which were comprehensible. The long and short prism patterns were then manually highlighted and classified for each sample. 100% assessment was achieved during double-blind assessment of these patterns.

The present technique is similar to the Verifinger software (Neurotechnology, Laisves pr. 125A, Vilnius, LT-06118, Baltic, Lithuania) used for the identification of fingerprints. The creation of a database from these images would make electronic storage possible for identification. The only drawback in the present method is the lack of automated software for a match from a database.

Attempts have been made to deduce enamel patterns using Verifinger[®] software in the past. However, patterns obtained by this software does not resemble the actual topography of enamel surface as in the SEM. The application of biometrics using Verifinger[®] software is further not convincing as it is designed to recognize ridges on the finger surface, such a presentation is absent on teeth, hence makes teeth inappropriate to serve as a template for recording of minutiae, which are basic units in dermatoglyphics.^[14–16]

The present study, thus, shows an avenue on designing of exclusive algorithms by attributing geometrical values for establishment amelogyphics as a reliable tool in personal identification and maintaining identification records. Amelogyphics can be proved to be a potent tool in maintaining personal identification records as it is unique, nonintrusively acquired and can be stored as an easily transmittable form of a database.

As the concept of amelogyphics is in its nascent stage, the manual method adopted for patterns recognition in the present study is bound to have few limitations too. The prism patterns are two-dimensional descriptions, and hence, there may be far more variability in shape and alignment than in the schematic diagrams. However, limitations can certainly be conquered with a more channelized and digitized approach in creating recognition patterns. It is clearly evident from the present study that amelogyphics are a sustainable and value-addition tool in the armamentarium of a forensic odontologist. The ease of obtaining prints, the high degree of reproducibility and the specificity of individual patterns parallel dermatoglyphics and should certainly serve as a legally acceptable form of identification.^[17]

The study also highlights that a new set of algorithms need to be established to record teeth patterns. Existing softwares for recording fingerprint patterns cannot be applied to record enamel prints.

Limitations

The small sample size of ($n = 10$).

Conclusion

Amelogyphics is a science with sound scientific basis. Currently, the science is in an evolving stage. The development of exclusive software would lead to the establishment of more authentic and reproducible database for personal identification in the field of forensic odontology.

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Conflicts of interest

There are no conflicts of interest.

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