#### **Review Article**

# Ameloglyphics- An Advancement in Forensic Science

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## **ABSTRACT**

The human teeth are considered as hard tissues similar to finger impressions (trustworthy means in a body found before decay or marring). Forensic dentistry is a comparatively young science that employs the awareness of dentists to assist the legal system. It has recognised itself as a vital science in medical and legal problems, particularly in the areas of individual recognition, sex determination, and age approximation. Teeth are highly repellent to surrounding influences like erosion, dehydration, putrefaction, and they can be utilised as criminal proofs. Skeletal remains, particularly teeth, can be used to identify people in crimes and catastrophic disasters. Enamel rod end motifs have recently become prominent as a subject of study. Ameloglyphics is a phrase that has been used to describe the investigation of these prints. The tooth imprints are one of a kind with differences between teeth from different people as well as within the same person. In terms of criminal dental recognition, mostly when it comes to recognising preys of man-made disasters, such as multiple casualties in army conflicts and wars, new and applicable methods of recognition and validation are urgently needed. This review article focuses on the rationale for employing enamel rod end patterns, as well as the methods for collecting them and identifying their sub-patterns and further suggests these tooth prints as analogues for fingerprints in deceased identification in disasters, crimes and other tragedies.

# INTRODUCTION

Before the 1960s, when the first official constitutional program in forensic dentistry materialised in the United States of America (USA) at the Armed Forces Institute of Pathology (AIFP), the field of forensic dentistry was largely in its infancy about research and development. The teeth and their supporting structures were able to provide a wealth of trustworthy information not only to the dentist but also to security agencies and other forensic investigating teams because their valuable contribution to the field of "forensic odontology" greatly expanded its branches [1]. According to Federation Dentaire Internationale (FDI), forensic odontology has been defined as that branch of dentistry that, in the interest of justice, deals with proper handling and examination of dental evidence and with the appropriate evaluation and presentation of dental findings [2]. Dr. Oscar Amoeda also referred to as the father of forensic odontology established forensic dentistry as a distinct field. The fundamental basis of forensic odontology is that no two mouths are the same [3]. Forensic dentistry processes, reviews, evaluates and presents dental evidence to contribute scientific facts and objective data to legal procedures. A dentist is actively involved in forensic odontology's many purposes, such as determining the age and sex of victims, personally identifying unidentified human remains, analysing bite marks as evidence, taking part in mass disasters, analysing lip prints, providing evidence in child abuse, and civil and criminal litigation [4]. Forensic odontologists in criminal investigations are therefore vital to the identification of human deceased. Dental evidence is emphasised in forensic odontology to identify victims and suspects [5]. Numerous forensic dental techniques can be used in circumstances where fingerprint identification is complex and visual recognition is not possible. This branch primarily uses a person's dentition to identify them. Rugoscopy, chieloscopys, tooth impressions, bite marks, radiography, photographic investigations, and molecular techniques are techniques used in forensic dentistry [6].

# **ROLE OF TEETH IN FORENSIC DENTISTRY**

Although DNA analysis is the method of choice for identifying humans, it is impossible in circumstances when the victims have

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been extensively mutilated. Based on the individual's state, teeth are helpful for identification in some instances [7]. The human dentition is used as a fingerprint surrogate. The human body's teeth are its most indestructible component since they can withstand calamities and serve as trustworthy forms of identification in severely burned remains [8]. The four main components of the teeth include enamel, dentin, pulp, and cementum, with enamel and dentin being the most calcified [9]. The term "tooth print" refers to enamel rods and patterns. "Ameloglyphics" is the investigation of these enamel rods and their patterns (tooth prints). After examining the rod end patterns, Manjunath K et al., came up with the word "ameloglyphics," which is similar to "dermatoglyphics" [9].

#### Enamel

Enamel's structural unit is its rods or prisms. Enamel rods on the tooth's exterior surface protrude from the dental-enamel junction [10]. Each tooth is unique from other teeth because of the numerous enamel rods that make up each one. Perikymata is the gradual enamel rod pattern seen while examining an object at a macroscopic scale [11]. Diverse patterns of enamel rod end can be seen on the tooth surface due to the different directions that enamel rods run in. Enamel rods are arranged in the key-hole or paddle-shaped patterns with rounded heads and narrow tails, according to studies using an electron microscope. Although there are many other ways that rods can be arranged, it is common to see rods with their heads close to the occlusal and incisal surfaces and their tails close to the cervical surfaces [12]. The tail of the previous row's enamel rod is assumed to be the substance that fills the space between the two rods in one row. The rods have a length of 9 µm and a width of 4-5 µm. Enamel rods' diameter grows in a ratio of 1:2 as they move from the dentinenamel junction to the tooth surface [13].

#### **Orientation of the Enamel Rods**

Usually, the enamel rods are positioned at a right angle to the dentin surface. Enamel rods in the cervical and middle third of deciduous teeth are horizontally oriented. They become more oblique and nearly vertical on the cusp tip as they approach the occlusal and incisal thirds. In the occlusal and middle thirds, the arrangement of enamel rods is similar to the arrangement in the deciduous teeth, but in the cervical third, the rods of enamel exhibit a rootward orientation or pass in an outward direction [14]. Due to the wavy arrangement and oblique direction, the length of enamel rods is greater than the thickness of enamel. It is different in different areas of the crown; it is longer in the cuspal area, which is a thicker portion, and shorter in the cervical area, which is a thinner portion [Table/Fig-1]. The crown is different in that it is longer in the cuspal area, which is a thicker portion, and shorter in the cervical area, which is a thinner portion. The enamel rod prisms are thought to be shaped as three distinct patterns, with pattern one consisting of circular prisms, pattern two consisting of parallel rows of prisms, and pattern three consisting of prisms arranged in one row so that the tail of the prism lies in between the two heads of the subsequent row, creating a key-hole pattern [Table/Fig-2] [15].





# METHODS FOR RECORDING ENAMEL RODS OR PRISMS

Among the most crucial stages in acquiring a tooth print in ameloglyphics, recording enamel rod endings on tooth surfaces with the appropriate material to completely and accurately replicate the enamel rod end patterns for personal identification is the most important. Enamel rod patterns on its surface can be captured a replicated using cellulose acetate film, cellophane tape, light body imprint compound, or metal-shadowed colloid ion film [16].

#### **Acid Etching**

The mineral composition of the enamel surface is eliminated during acid etching. The smear layer is removed during etching, which causes the surface enamel to be distributed unevenly. The acids utilised for this method are 10% citric acid, 10% phosphoric acid, 10% maleic acid, 2.5% oxalic acid, and 2.5% nitric acid; the most popular form is the gel form of the 10% orthophosphoric acid [17]. Demineralisation is selective in specific microregions because of morphological modifications in the arrangement of the prism crystals. This process removes 10  $\mu$ m of the enamel surface, creating a porous layer 5-50  $\mu$ m deep. The degree to which the

rods are angled can affect demineralisation; the angle is greater at the prism's head or periphery. Prism patterns can be divided into two types: type one displays a honeycomb pattern, while type two displays a cobblestone look. Type one and type two mixed patterns, pitted enamel surfaces that resemble unfinished puzzles, flat and smooth surfaces, and other less prevalent patterns have all been seen. The way enamel reacts to etching depends on the type of acid used, the acid concentration, the length of time spent etching, the rinsing time, whether or not prior instrumentation of enamel is done before etching, the chemical content of enamel, and its condition. Surface instrumentation, patient age, and external factors contribute to minor variations in enamel characteristics and impact the conditioners' ability to demineralise correctly [18]. This shows that further studies need to be conducted to study the time of etching and other factors.

#### **Acetate Film Technique**

Having been etched and processed on an acetate film, a peel is a representation of a mineral surface. Peeling has the benefit of being a straightforward, inexpensive, and quick approach to creating replicas of dental hard tissues. To analyse the cellular components of fossil plants, paleobotanists initially devised this technique. The method was altered to investigate dental hard tissues because of their unique structural composition. Under a light microscope, the peel is inspected. The entire pattern and sub-pattern of the enamel rod end can be precisely captured using acetate peel instead of cellophane tape and rubber base impression compound. Therefore, the acetate peel technique is regarded as the best way to capture enamel rod ends on tooth surfaces. No incomplete patterns or empty spaces are observed in this procedure. Using this method, subsequent marks made on the same tooth's same region will replicate the same pattern and sub-patterns [19]. The drawback of the peel technique is that if the cellulose acetate peel is not correctly adapted to the uneven tooth surface, it becomes difficult to record the surface enamel rod endings of the complete teeth [9]. In a study by Manjunath K et al., the acetate peel technique was used to examine 30 teeth. The research revealed several sub-patterns, including wavy-branched, wavy-unbranched, linear-branched, linearunbranched, whorl-open, whorl-closed, loop, and stem-like. The thirty dental prints found did not resemble one another [9].

### **Cellophane Tape Technique**

The tooth sample is cleaned and sized before using the cellophane tape method. The labial surface is cleaned and allowed to air dry. Later, 37% orthophosphoric acid is used to etch the labial surface for 2 minutes. After washing, the etched surface is allowed to air dry. A cut piece of cellophane tape is applied to the dried area. A tiny amount of rolled cotton is used to aid in better adaptation without applying pressure. Cellophane tape is carefully pulled off, transferred to a slide, and examined with an Olympus CX 21 microscope [20]. To determine whether a person's dental prints can be utilised for personal identification after exposure to strong acid, Juneja M et al., performed a study using the cellophane tape approach. Twenty extracted teeth yielded a total of 90 dental impressions; nevertheless, the study's findings showed that none of the 20 tooth impressions were identical to one another, either between individuals or within individuals [20].

#### **Automated Biomatrix**

The statistical examination of biological data is known as "biometrics" [21]. It is a technique that identifies or authenticates a person in a digital value using their biological, physical, or behavioural traits. When the system identifies the patterns submitted for identification, this is referred to as positive identification. Facial recognition, iris scanning, and fingerprint verification are some biometric-based identification and verification methodologies used in forensics. The

information is stored in automated systems and software that can reliably identify people [22].

Data should be easily transmissible, easily recognised by all, and obtained without intervention in a perfect automated system. It should also be highly distinctive to each individual [23-25]. The analysis of fingerprints can be done using a variety of software programs. The Verifinger® standard SDK version 5.0 software was produced by Neurotechnologia in 1998. The Verifinger SDK is researched for integrators and developers. It allows for the development of biometric software for the Windows, Linux, and Mac OS X operating systems. Integrating Verifinger into a customer's security system is simple. Any scanner, database, and user interface can be used with the integrator because it manages all of the SDK's input and output data. It deposits a picture that mimics the minutiae in the form of an outline using a particular collection of fingerprint points (called minutiae). Ramenzoni LL and Line SR were the first to use fingerprint identification and verification software (VeriFinger Demo 4.2, SDK/Fingersec) to evaluate the distinctiveness of Hunter-Schreger bands for personal identification [26].

Additionally, all surface patterns of a specific tooth with a particular ID number previously saved in the database can be identified with the help of the Verifinger software. The gold standard for recreating enamel rod-end patterns is VeriFinger software version 5.0. It can also be duplicated precisely as well. VerFinger 10.0 Standard SDK and VeriFinger 10.0 Extended SDK are the latest versions, but research for dental print copying has not been confirmed [26]. Ramenzoni LL and Line SR conducted a study at Piracicaba Dental School, State University of Campinas, SP, Brazil, to identify the patterns of Hunter-Schreger bands with a sample size of 245 lower central incisors using an automated biomatrix technique. They also performed an in-vitro analysis of 30 teeth directly from the mouths of individuals and found that the tooth print on one side is different from its homologous sides. This indicates that tooth prints are specific and unique for each individual. In the article, they concluded that an automated biomatrix could be used as a biometric-based parameter for personal identification in automated systems [26].

#### **Rubber Base Impression Material Technique**

This method involves doing research using a light body impression substance. The light body's base paste and catalyst are combined evenly to create the correct consistency before being applied to the tooth's conditioned surface [27]. The imprint is carefully peeled and transferred to a glass slide when it has had time to set. After that, it is examined using an Olympus CH 20i stereomicroscope. Because it is not translucent, it cannot be seen with a light microscope. The benefits of employing light body material are its light consistency, reduced viscosity, and contribution to the accurate recording of details. Using this method, Manjunath K et al., conducted a study in which 30-rod ending prints were analysed. There were empty areas and incomplete patterns. The same tooth was used for the second impression, empty spaces from the first imprint revealed enamel rod patterns and sub-patterns, and empty spaces were moved to different locations. The Verifinger program could not match a specific tooth's enamel rod end pattern to its previously recorded and database-stored unique identification number [18].

# AMELOGLYPHICS AND THE RELATIONSHIP BETWEEN DENTAL CARIES

Dental caries is a chronic microbial disease that affects the hard tissues of the mouth [8]. It permanently damages the tooth and changes its structural makeup. It may be the only illness that affects people consistently and irrespective of their location, age, gender, financial level, and other characteristics. It is also the most prevalent dental issue in the entire world. The causes, pathophysiology, possible therapies, and, most crucially, how to avoid dental caries must all be thoroughly understood by clinicians. Examining enamel

rod end patterns can help you better comprehend the enamel's surface structure. This structural examination may be incredibly beneficial in identifying and avoiding dental caries because dental caries that affect smooth surfaces of teeth may go undetected until a substantial defect develops [28]. In a study by Girish HC et al., enamel rod patterns were used to differentiate between 30 carious and 30 non carious teeth. The cellophane tape method was utilised. The outcomes demonstrated that the enamel rod pattern was unaltered in the teeth affected by caries. Unaffected teeth did not exhibit any distinct patterns either [29].

### AMELOGLYPHICS IN MASS DISASTER

A mass disaster is a chaotic incident brought on by a destructive force that causes many casualties and necessitates identification. The vast majority of victims of a catastrophic disaster are difficult to identify due to extreme mutilation, charring, and decay. Traditional identifying techniques, such as passwords, fingerprints, DNA testing, and pictures, cannot be utilised under such circumstances [30]. The body's strongest and chemically most stable structure, teeth, are resistant to postmortem deterioration and damage and are protected from trauma. Each person's enamel teeth prints are distinctive. They may be helpful for identity verification. In forensic dentistry, ameloglyphics is a growing field. The permanence and reproducibility of tooth prints determine their value even though they are personal to each person. Therefore, more research is needed to examine the use of ameloglyphics for individual identification in major catastrophes [31]. Vanezuela A et al., conducted a study in 2000 on a bus accident case in Spain where 28 people were burn victims. They conducted postmortem procedures for identification which included radiographs, external examination, photographs as well as dental examination. Dental identification was established in 57% of the cases. When victims were less than 20 years of age, the success rate of identification by dental methods was higher (76% of victims in this age group). The assessment of dental age allowed the establishment of identity of four victims. They concluded that dental and radiographical examinations were of utmost helpful in post mass disaster identification [32].

Manjunath K et al., conducted a study to study the effectiveness of various materials for recording enamel prints for personal identification, wherein he recorded enamel rod endings from 30 extracted teeth from the same area of the same tooth twice using acetate peel technique, cellulose tape, rubber base impression material. Enamel rod ending photomicrographs were taken, and Verifinger standard SDK version 5.0 software was used to analyse the results. Statistics were used to examine the minutiae scores of all enamel rod end patterns made using these three imprint materials. The 'y' found that the acetate peel technique revealed complete enamel rod end patterns, and the software was able to identify the particular tooth with the same previous identification number in subsequent recordings as well. However, Cellophane tape and light body rubber-base impression material imprint showed incomplete enamel rod end patterns. The software failed to identify the particular tooth with the same previous identification number in subsequent recordings. Statistical analysis revealed that cellulose acetate film recorded more minutiae points compared with the other imprint materials. Hence, acetate peel technique is a reliable method for identifying enamel rod end patterns as compared to other techniques [33].

The advantages and disadvantages of various techniques for recording enamel rods or prisms have been tabulated in [Table/Fig-3] [9,18,20,25,27]. The recording procedure of enamel prints have been illustrated in [Table/Fig-4].

This research suggested that ameloglyphics can be a valuable technique for identifying a person even in the presence of diversity, such as burns and acid attacks. Ameloglyphics offer additional investigative avenues for person identification during forensic Harshita Ramdev Ganeshani et al., Ameloglyphics in Forensic Science

Techniques	Advantages	Disadvantages	
Acid etching	Various prisms patterns can be identified	As this technique depends on various factors like etching time, surface instrumentation, it can alter the results to be obtained	
Acetate peel technique	The entire pattern and sub-pattern of the enamel rod end can be precisely captured using acetate peel. No variations in minutae points is seen	The drawback of the peel technique is that if the cellulose acetate peel is not correctly adapted to the uneven tooth surface, it becomes difficult to record the surface enamel rod endings of the complete teeth.	
Cellophane tape and rubber base impression techniques	Both the techniques are easy to conduct	The position enamel prints of one tooth when compared to the previous position of enamel prints on same tooth differs and biometric analysis fails to identify the tooth. Variation in minutae points seen.	
Automated biomatrix	It is software-based technique; hence data is saved can be used whenever needed.	Software are not reliable and need to be updated.	

[Table/Fig-3]: Advantages and disadvantages of techniques [9,18,20,25,27].



investigations, especially in cases of severe burns and fatalities. Ameloglyphic personal identification is a reliable and secure method. Clinical application of ameloglyphics [28,32].

In the present time, ameloglyphics is used for various purposes. The application is widespread, including predilection of caries; in forensic identification, where the bodies are completely mutilated, the teeth can be used to identify the individual; in mass disaster cases, to identify and differentiate people. It can be used for individual identification of occupational hazards. The exclusive nature of our dental anatomy and the placement of custom restorations ensure accuracy when the techniques are appropriately employed. Dental records of patients can also be useful in legal procedures during the identification process. Studies on ameliglyphics have been tabulated in [Table/Fig-5] [20,28,33,34]. Although enamel is the hardest tissue of the body, it does undergo micro and macro abrasions in daily life. Hence, the effect of daily life activities like tooth brushing needs to be determined. The direction of enamel rods varies according to the thickness of enamel which is different in different areas of the tooth, so further studies should be conducted to verify the relationship between the direction of rods and the thickness of enamel. Ameloglyphics is still in its initiation stage and depends upon its

Author	Place and year of study	Studies	Result
Juneja M et al., [20]	Uttar Pradesh 2016	Ameloglyphics: a possible forensic tool for personal identification following high temperature and acidic exposure	After being subjected to acid or heat, tooth imprints from a similar tooth were reproducible and exhibited an extreme likeness to the primary tooth print of that specific tooth.
Manjunath K et al., [33]	Andhra Pradesh, 2012	Analysis of enamel rod end patterns on the tooth surface for personal identification- Ameloglyphics	The biometric analysis demonstrated intra- individual as well as interindividual differences. The male and female patients had different enamel rod end designs.
RG Sugunakar et al., [28]	India, 2014	Ameloglyphics: can it aid in forensic identification	The samples revealed distinct enamel end patterns that were both exclusive to a distinctive personality and unique to a tooth
Christopher V et al., [34]	Karnataka, 2017	Can dead man tooth tell tales? Tooth prints in forensic identification	In many situations, comparing two tooth prints collected from a person at two intervals revealed a resemblance, with the wavy pattern tooth print being the most common. However, the same prints displayed differences when compared to other people's prints. They also discovered that most people with whorl pattern fingerprints had wavy pattern tooth prints, while only a few people with loog type fingerprints had linear pattern tooth prints.

[Table/Fig-5]: Historical view of studies conducted [20,28,33,34].

reproduction and permanency; hence further studies need to be conducted to determine the efficacy of this technique [35].

# CONCLUSION(S)

In forensic science, the distinctiveness of tooth prints could be used as a valuable recognition method. As stated by the studies conducted, tooth prints seem to be exclusive to a person, with differences among those of various people as well as those of similar persons, and these prints are also repeatable even when the tooth is exposed to damaging conditions such as extreme temperatures and an acidic circumstance. Ameloglyphics significance as a criminal learning instrument for individual recognition is based upon its permanence and its reproducibility. These two characteristics of teeth prints should be studied in a bigger cohort. However, in all the articles reviewed there has been no mention of ethnic variation in rod patterns, which needs to be studied further. Further research on topics such as how daily life activities affect enamel rods, how developmental defects affect enamel rods, and the association of tooth prints with sex and age need to be conducted to validate the reliability of ameloglyphics.

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