# ORIGINAL ARTICLE

# Efficacy of various materials in recording enamel rod endings on tooth surface for personal identification

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# **Abstract**

Aim: To analyze efficacy of cellulose acetate film, cellophane tape, and light body impression material in recording enamel rod endings on tooth surface for personal identification. Materials and Methods: Surface enamel rod endings of 30 extracted teeth were recorded from the same area of the same tooth for two times using cellulose acetate film, cellophane tape, and light body impression material. Photomicrographs of enamel rod endings were taken and subjected to Verifinger standard SDK version 5.0 software for analysis. Minutiae scores of all enamel rod end patterns obtained using these three imprint materials were statistically analyzed. Results: Cellulose acetate film imprint showed 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. Cellophane tape and light body rubber-base impression material imprint showed incomplete enamel rod end patterns and 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 number of minutiae points compared with the other imprint materials. Conclusion: Cellulose acetate film is a reliable material for recording enamel rod endings on tooth surface for personal identification compared with cellophane tape and light body rubber-base impression material.

**Key words:** Ameloglyphics, cellulose acetate film, cellophane tape, rubber-base materials

# Introduction

Personal identification is becoming very important in the present world. It is usually achieved by the use of passwords, physical tokens, photographs, iris and dental patterns, fingerprints, and more recently, DNA analysis. But, these identification methods have certain limitations

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and may not be efficient when bodies are decomposed, burned, or in cases where only small fragments of calcified tissues are left. Dental hard tissues gain importance in identification based on the condition of the deceased. Teeth can withstand extreme temperatures and resistant to postmortem decomposition. Moreover, restorative materials used by the dentist for restoring teeth are also resistant to postmortem destruction to certain extent. Therefore, the use of dental evidence is the method of choice in establishing identity of badly burned, traumatized, decomposed, and skeletonized remains.<sup>[1]</sup>

Enamel is the hardest calcified tissue in the human body. [2] It consists of undulating and intertwining enamel rods emerging from dentinoenamel junction till external tooth surface. [3] Macroscopically, incremental pattern of enamel

rods is exhibited on tooth surface as perikymata,[4] but microscopically, groups of enamel rods run in unique direction, which differ from adjacent group of enamel rods and results in forming different patterns of enamel rod endings on tooth surface. [5,6] Manjunath et al. recorded the enamel rod endings on tooth surface using cellulose acetate peel technique and coined the term "ameloglyphics." Ameloglyphics is the study of enamel rod end patterns on tooth surface. Enamel rod end patterns were unique for each tooth in an individual and may be used as an adjunct with other methods for personal identification. This technique is simple, inexpensive, and rapid method which can be performed by even a dental auxiliary staff. Usually, this method of personal identification can be recommended for those individuals working in dangerous occupations such as fire fighters, soldiers, jet pilots, divers, and people who live or travel to politically unstable areas prior to their assignments and must be updated periodically to overcome the enamel loss due to wear and tear. [7] Nidhi et al. recorded enamel rod end patterns on tooth surface using cellophane tape and revealed that enamel rod end patterns seem to be unique to an individual; however, they not only found dissimilarities between different individuals, but also within the same individual.[8]

In 1998, Neurotechnologija developed Verifinger SDK identification software for biometric system integrators. [9] Ramenzoni and Line used Verifinger SDK v4.2 software for automated biometric study of Hunter-Schreger bands (HSB) in enamel for personal identification. [10] Manjunath *et al.* showed that Verifinger SDK v5.0 software is a reliable biometric tool for the analysis of enamel rod end patterns in ameloglyphics. [11] The aim of the present study is to analyze efficacy of cellulose acetate film, cellophane tape, and light body impression material in recording enamel rod endings on tooth surface for personal identification.

#### **Materials and Methods**

#### Sample

Thirty extracted permanent teeth were collected from the Department of Oral and Maxillofacial surgery in our institution. Soon after extraction, teeth were cleaned using normal saline to remove all blood stains and saliva. Later, all the teeth were stored in 10% formalin at room temperature. Teeth with hypoplasia, dental caries, regressive alterations, fractures, and/or restorations were excluded.

# Procedure

All the 30 extracted teeth were cleaned using an ultrasonic scaler and polished. A  $0.5 \times 0.5$  sqcm area on the buccal surface (middle third) of the teeth was conditioned using 10% orthophosphoric acid for 20 seconds and then washed with water and air-dried to avoid contamination. The middle third area of buccal tooth surface was selected in

our study since it is the least prone area for fracture and development of dental caries when compared with other areas. Enamel rod endings on the middle-third area of the buccal tooth surface of all teeth were recorded using cellulose acetate film, cellophane tape, and light body rubber-base impression material.

#### Cellulose acetate peel technique

A thin layer of acetone was applied over a small piece of cellulose acetate film and placed immediately over the conditioned surface of the teeth without any finger pressure for 15 minutes. The acetone dissolves a layer of cellulose acetate and the dissolute settles down along the irregularities on the enamel surface. The film was gently peeled after 15 minutes. The peeled strip was placed in the center and perpendicular to the length of a clear glass slide and observed under an Olympus CH 20i light microscope (Zenith engineers, Agra, India). Photomicrograph of the imprint was obtained at 40× magnification in the microscope using Nikon 5200 digital camera (Nikon Inc, Melville, USA) in 1.4× digital zoom. The photomicrograph was then cropped at the center to 2000 × 1500 pixels dimension using Microsoft picture manager software (Microsoft Corp., Redmond, WA, USA) [Figure 1].

# Cellophane tape technique

A portion of extended cellophane tape was applied over the conditioned area without finger pressure. A small piece of cotton roll was applied over the same for better adaption of the cellophane tape. The cellophane tape was then immediately pulled off gently and placed on a clean glass slide and observed under Olympus light microscope. Photomicrograph of the imprint was obtained at 40× magnification in the microscope using the Nikon 5200 digital camera at 1.4× digital zoom. The photomicrograph was then cropped at the center to 2000 × 1500 pixels dimension using the Microsoft picture manager software [Figure 2].

#### Rubber-base impression material technique

The catalyst and base of light body rubber impression material were evenly mixed for appropriate consistency and immediately placed on the conditioned surface. Light body type impression material was used because of its thin consistency and less viscosity, which helps record even minor details more accurately. After the setting of the impression material, the replica was carefully peeled and placed on a clean glass slide and observed under the Olympus CH 20i stereomicroscope (because rubber-base impression material is not a translucent material to view under light microscope). Photomicrograph of the imprint was obtained using the Nikon 5200 digital camera. The photomicrograph was then cropped at the center to 2000 × 1500 pixels dimension using the Microsoft picture manager software [Figure 3].



Figure 1: Cellulose acetate film replica of enamel rod endings recorded from tooth surface



Figure 3: Rubber-base impression material replica of enamel rod endings recorded from tooth surface

#### Biometric analysis

All cropped photomicrographs were subjected to Verifinger standard SDK v 5.0 software program for biometric analysis. The software recognizes the patterns and sub-patterns of enamel rod endings from cellulose acetate film, cellophane tape, and rubber-base impression material replicas as a series of lines running in varying directions and memorized in the database with specific identification number and minutiae points on it. Minutiae points are specific identification points on enamel rod end patterns which are recorded by the software for further identification and verification. Minutiae points may be line endings, dot, very small lines, ponds, empty spaces between two lines, and branching/bisecting of two lines. Enamel rod end patterns recorded by the software can be stored in the computer for office record purpose in the form of black and white lines without minutiae points.

Enamel rod end patterns are recorded from the same area of the same tooth on two occasions using the same materials to check the reliability of the imprint materials in reproducing



Figure 2: Cellophane tape replica of enamel rod endings recorded from tooth surface

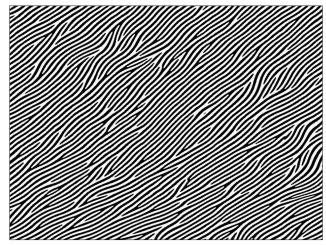


Figure 4: Enamel rod end patterns obtained from cellulose acetate film replica

the same pattern and sub-patterns of enamel rod endings on tooth surface.

# Statistical analysis

The minutiae scores on all the enamel rod end patterns of each tooth recorded using cellulose acetate film, cellophane tape, and light body rubber-base impression material were tabulated and statistically analyzed using Mann-Whitney test. A Mann-Whitney test reliability score of  $\leq\!0.05$  represents that two imprints compared were statistically different. Pearson Correlation and Kendall's tau correlation were used to correlate the minutiae scores between these materials.

# Results

#### Cellulose acetate peel technique

The cellulose acetate peel imprint of each of the 30 teeth composed of complete and unique patterns and subpatterns of surface enamel rod endings [Figure 4]. No

empty spaces or incomplete patterns were seen. This technique reproduced the same pattern and sub-patterns in subsequent imprints taken from the same area of the same tooth. The Verifinger software matched the enamel rod end patterns of all the teeth with specific identification number which was stored in the database in the previous recording. No significant difference in minutiae point locations and scores was observed in subsequent imprints taken from the same area of the same tooth.

# Cellophane tape technique

Upon analysis of the 30 enamel rod ending imprints, it was observed that each imprint composed of incomplete enamel rod patterns and sub-patterns. Empty spaces and incomplete patterns were seen [Figure 5]. On taking imprints again from same area of the same tooth, empty spaces of previous imprint showed enamel rod end patterns and sub-patterns and/or shift of empty spaces to other areas [Figure 6]. Minutiae point locations and scores were variable. The Verifinger software failed to match the



Figure 5: Enamel rod end patterns obtained from cellophane tape replica. Empty areas devoid of enamel rod end patterns are seen

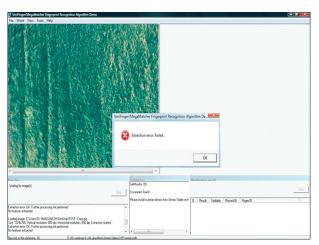


Figure 7: Verifinger SDK v6.0 software failed to extract enamel rod end pattern from light body rubber-base impression material replica

enamel rod end pattern of a particular tooth with specific identification number which was stored in the database in the previous recording.

#### Rubber-base impression material technique

Upon analysis of the 30 enamel rod ending imprints, the software was denied the extraction of patterns from 27 imprints [Figure 7], whereas three imprints showed empty spaces and incomplete patterns [Figure 8]. On repeating the imprints from same area of the same tooth, empty spaces of previous imprint showed enamel rod end patterns and sub-patterns and/or shift of empty spaces to other areas. The minutiae point locations and scores were variable. The Verifinger software failed to match enamel rod end pattern of a particular tooth with the specific identification number that was stored in the database in the previous recording.

# Statistical analysis

The mean minutiae scores of cellulose acetate film, cellophane tape, and rubber-base impression material



**Figure 6:** Enamel rod end patterns obtained from cellophane tape replica. Empty spaces of previous imprint showed enamel rod end patterns and sub-patterns and/or shift of empty spaces to other areas



Figure 8: Enamel rod end patterns obtained from rubber-base impression material replica

imprints were 363.3, 133.2, and 5.7, respectively. Pearson correlation and Kendall's tau correlation revealed that cellulose acetate film, cellophane tape, and rubber-base impression material imprints were not statistically correlated. Mann-Whitney test revealed significant difference in minutiae score between cellulose acetate film, cellophane tape, and rubber-base impression material imprints (P value  $\leq 0.05$  - significant difference,  $\geq 0.05$  - not significant difference) [Table 1].

# Discussion

Teeth have been extensively used as a source of information in forensic sciences, especially when the soft tissues cannot provide reliable information. [12,13] Recently, ameloglyphics have been proposed and considered as a highly reliable biometric-based procedure for personal identification.<sup>[11]</sup> Formation of enamel is a highly organized process in which the ameloblasts lay down the enamel rods in an undulating and intertwining path.[3] The regular change in their directions results in the optical phenomenon known as HSB. HSB appear as dark and light bands under reflected light, [14,15] a phenomenon that occurs because enamel prisms function like optic fiber when exposed to a directed source of light. [10,16] These undulated groups of enamel rods end on tooth surface in different directions and at different levels, which form specific patterns on tooth surface. The study of these enamel rod end patterns are called as ameloglyphics.<sup>[7]</sup>

Alkaya et al. used acetate peel technique to study dental structures in three-dimensional view, especially from fully mineralized enamel without routine decalcification, sawing, and mounting processes.<sup>[17]</sup> Manjunath et al. used a modified cellulose acetate peel technique for recording enamel rod endings on tooth surface and these patterns were intended for use in personal identification.<sup>[7]</sup> Gupta et al. used cellophane tape for recording enamel rod endings on tooth surface<sup>[8]</sup> and the patterns of enamel rod endings obtained by them were similar to our imprints recorded using the same material, which was incomplete and improper (in these recordings also, empty spaces and incomplete patterns were seen). On taking imprints again from the same area of the same tooth, enamel rod end patterns and sub-patterns in empty spaces seen in previous imprints and/or shift of empty spaces to other areas are seen. Yet, in this study, series of trials are conducted using cellulose acetate film, cellophane tape, and rubber-base impression materials to select the ideal material for recording enamel rod end patterns on tooth surface.

In our study, cellulose acetate imprints showed complete patterns and sub-patterns of enamel rod endings. No empty spaces and incomplete patterns were seen. No significant difference in minutiae point locations and scores was observed in subsequent imprints taken from the same area of the same tooth. This technique reproduced the same pattern and sub-patterns of enamel rod endings in subsequent imprints taken from the same area of the same tooth. Also, the Verifinger software identified all the enamel rod end patterns of a particular tooth with specific identification number which was stored in the database during previous recording. However, cellophane tape and rubber-base impression material imprints showed incomplete enamel rod end patterns. These patterns were not specific for any particular tooth. The Verifinger software failed to identify enamel rod end patterns of a particular tooth with specific identification number which was stored in the database during previous recordings; so, reliability of these materials to reproduce unique and complete enamel rod end pattern for personal identification is questionable. Of 30 rubberbase impression material imprints, the software was able to extract enamel rod end patterns from only eight imprints. The extracted enamel rod end patterns were incomplete and nonspecific for the particular tooth, which may be due to improper flow and consistency of the material for recording enamel rod endings on tooth surface. Statistical analysis reveals that cellulose acetate film imprint of enamel rod endings on tooth surface recorded the maximum minutiae points and more reliable material for recording enamel rod end patterns on tooth surface compared with cellophane tape and rubber-base impression material.

Cellophane tape contains polythene film with thin layer of adhesive material on one side. The adhesive material will be very viscous and have insufficient flow to record all enamel rod endings on the tooth surface. In contrast, the application of acetone dissolves the layer of cellulose acetate film and the dissolute settles down along the irregularities on enamel surface, which was actually intended to record the complete enamel rod endings on tooth surface even without applying any pressure. So, recording of enamel

Table 1: Minutiae scores and comparison of three imprint materials for recording enamel rod end patterns on tooth surface

Imprint materials	Mean	S D	Pearson correlation		Kendall's tau correlation		Mann-Whitney test
			Score	P	Score	P	
Cellulose acetate film Cellophane tape	363.3 133.2	10.3 5.4	0.040	0.834	0.055	0.680	0.000
Cellulose acetate film Rubber-base material	363.3 5.7	10.3 9.8	0.126	0.508	0.095	0.519	0.000
Cellophane tape Rubber-base material	133.2 5.7	5.4 9.8	0.032	0.867	0.010	0.945	0.001

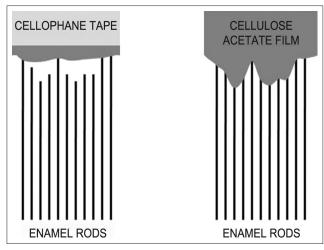


Figure 9: Schematic representation of enamel rod ends recording using cellophane tape and cellulose acetate film

rod end patterns using cellophane tape gives incomplete replicas of enamel rod endings compared with cellulose acetate film [Figure 9].

Cellulose acetate film technique is a simple, inexpensive, accurate, and rapid method for recording enamel rod endings on tooth surface, but it is difficult to record complete tooth surface enamel rod endings using this technique because of improper adaptations of cellulose acetate film on tooth surface irregularities. Therefore, regular implementation of this technique may be developed further by use of fiber-optic laser scanner that would scan the complete facial surface of multiple teeth and software dedicated to analysis of enamel rod endings on tooth surface. Also, the familial and geographical variation of enamel rod end patterns should be revealed in further studies.

#### Conclusion

In ameloglyphics, recording of enamel rod endings on tooth surface using proper material is an important step for reproducing complete and accurate enamel rod end patterns for personal identification. In our study, cellulose acetate film reproduced the complete and accurate enamel rod end patterns compared with cellophane tape and light body rubber-base impression material; so, cellulose acetate film is considered as a reliable material for recording enamel rod endings on tooth surface for personal identification.

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