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Research Article

Rosin's Edge in Forensic Odontology: A Staining Insight

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Abstract

Introduction: Forensic odontology is a specialized field at the crossroads of dentistry and law, focusing on the analysis of dental evidence in legal investigations. Due to their resistance to decay and environmental degradation, teeth serve as a vital resource in age estimation and gender determination. Various techniques, including microscopic examination of the incremental lines in enamel and cementum, neonatal lines, and dentin translucency, are commonly employed for forensic analysis. To enhance the accuracy and ease of examination, there is an increasing demand for high-quality, non-demineralized tooth sections that are durable, easy to handle, and maintain uniform thickness.

Aims and objectives: 1. Evaluate the effectiveness of the Rosin stain in identifying incremental lines in hard dental tissues. 2. Compare Rosin stain with Haematoxylin and Eosin stain as well as with unstained sections.

Materials and methods: Sixty sound teeth were included in the study. Thin longitudinal sections of 2 mm were prepared using a diamond disc, and ground sections of 25 µm were prepared on Arkansas stone. The sections were divided into three groups: (A) sections stained with Rosin, (B) sections stained with H&E, and (C) unstained sections. The longitudinal ground sections were examined under both the Light Microscope and the Phase Contrast Microscope. The Microstructures of the teeth, such as incremental lines of enamel, dentin, and cementum, were assessed.

Result and conclusion: Rosin-stained ground sections observed under phase contrast microscopy provided better visualisation of dental microstructures than unstained or H&E-stained ground sections, suggesting Rosin enhances the identification of incremental lines in forensic dental analysis.

More Information

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Keywords: Incremental lines; Light microscope; Phase contrast microscope; Age estimation; Rosin; Haematoxylin; Eosin

Introduction

Forensic odontology, also known as forensic dentistry, involves the application of dental expertise to criminal and civil law, assisting law enforcement agencies and the criminal justice system in legal investigations. The Federation Dentaire International (FDI) defines Forensic odontology as "that branch of dentistry which, in the interest of justice, deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings [1].

Teeth are among the hardest and most durable tissues in the human body, highly resistant to decomposition. Forensic dental identification plays a crucial role in identifying human remains, particularly in cases where postmortem changes or traumatic injuries limit the effectiveness of other methods. It is especially significant in criminal investigations and mass disasters.

Age determination is a key component of forensic medicine, aiding both in identifying bodies and crime-related investigations. The gradual structural changes in teeth throughout a person's lifetime serve as the foundation for age estimation. By analyzing the enamel, dentin, and cementum as the primary components of teeth, it is possible to estimate the chronological age of unidentified individuals [2].

In 1950, Gösta Gustafson proposed an age estimation method based on morphological and histological changes in teeth. This method assessed several age-related dental changes, such as attrition (A), secondary dentin deposition

(S), apical migration of periodontal ligament attachment (P), cementum apposition at the root apex (C), root resorption at the apex (R), and root dentin translucency (T) [3]. Various techniques are used to study dental microstructures, including-

- Preparation of undemineralised thin ground sections
- Decalcification followed by staining with H&E, basic/ acidic fuchsin, or Toluidine blue, and subjected to histological examination.

There is a growing need for high-quality, non-demineralized tooth sections that are easy to handle, maintain uniform thickness, and allow clear observation of intact microstructures. These sections should also be preserved for extended periods and be suitable for microscopic techniques such as light microscopy and advanced phase contrast microscopy for successful employment and to enhance the appearance of dental microstructures. Rosin, or colophony, is a brittle, solid resin derived from conifer trees. Its color ranges from nearly colorless to various shades of yellow, brown, and black. The two main types are gum rosin, left after distilling tapped tree resin, and tall oil rosin, derived from wood pulping, respectively. Gum rosin is a versatile raw material widely used in the production of resins, curing agents, surfactants, biomedical materials, elastomers, coatings, adhesives, sorbents, and catalysts [4].

Hence, this study aimed to explore the Rosin staining technique to enhance the visualization of dental microstructures in ground sections of undemineralized teeth, aiding age estimation in forensic odontology.

Materials and methods

This study was conducted over a period of two months. The sample size was determined based on available resources. A total of 30 extracted human teeth, free from caries and periodontal diseases, were collected and preserved in 10% neutral buffered formalin. The teeth were thoroughly rinsed under tap water before sectioning. Thin longitudinal sections measuring 2 mm were prepared using a diamond disc, and ground sections of 25 µm thickness were obtained by grinding the samples on an Arkansas stone. The sections were then divided into three groups: (A) section stained with Rosin, (B) section stained with Haematoxylin and Eosin, and (C) unstained section. The longitudinal ground sections were examined and compared under both the Light Microscope & Phase Contrast Microscope.

Staining method

Staining procedure for Rosin:

1. Preparation of Staining Solution

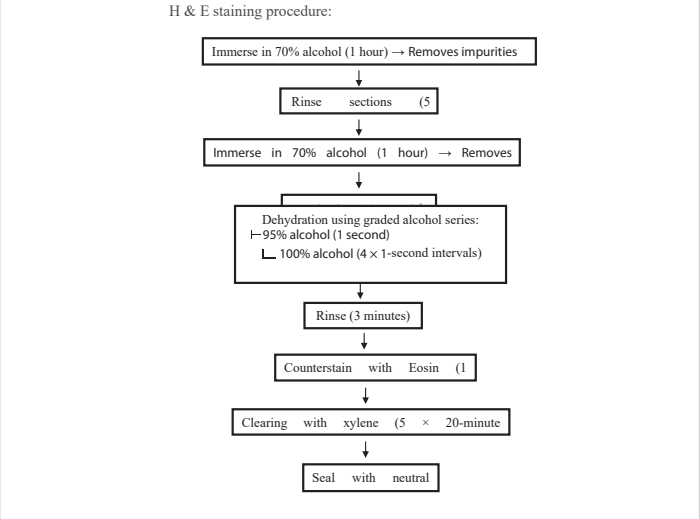
- Mix Rosin (100 g), Xylene (50 ml), and Glycerol (15 ml)
- Heat at 100 °C in an oven until fully melted

2. Staining Process

- Immerse tooth sections in melted Rosin
- Duration: 5 minutes
- Ensures rapid staining

3. Mounting & Examination

- Place stained sections on glass slides
- Mount using neutral gum
- Examine under: Light and Phase Contrast Microscope



For unstained sections, after mounting with DPX, the ground sections were examined under both light and phase-contrast microscopes (Figures 1,2).

The sections were graded from 0 to 3 based on the differentiation and tissue contrast observed using various methods.

0	Poor
1	Satisfactory
2	Good
3	Excellent

Figure 1: Akansas stone for thinning sections.

Figure 2: Longitudinal ground section of teeth.

Results

A total of 60 ground sections of healthy human teeth were examined using a compound light microscope and a phase contrast microscope to analyse dental microstructures. These microstructures were compared based on different staining methods, as shown in Figures 3,4. The obtained data were subjected to statistical analysis, including ANOVA and Pearson's t-test to assess correlation among variables. The enamel, dentinal tubules, cemental annulations, and other specialized tooth structures were more distinctly visible in the rosin-stained sections compared to those stained with hematoxylin and eosin or the unstained sections [5]. When comparing the compound light microscope and phase contrast microscope, a significantly higher correlation was observed using the phase contrast microscope for evaluating dental structure (Tables 1,2).

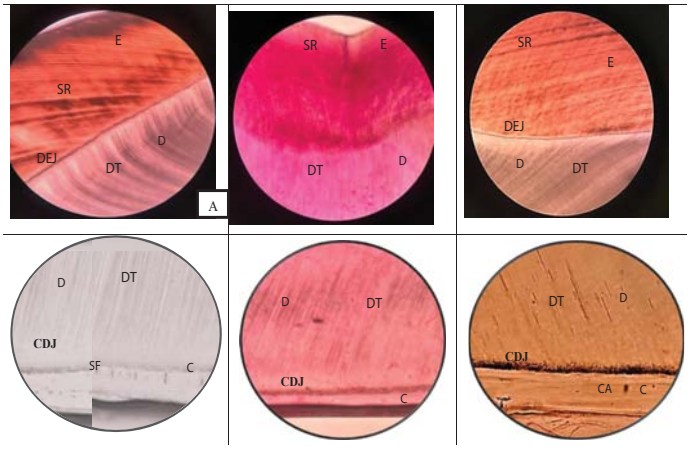


Figure 3: Photomicrograph of non-demineralized ground sections under Compound Light Microscope(10x) showing- Enamel(E); Striae of Retzius (SR); DEJ; Dentin(D); Dentin tubules (DT); Cementum(C); Cemental annulations (CA); Sharpey's fibres; Cementocytes; Tome's Granular layer; Cemento-dentinal Junction (CDJ).

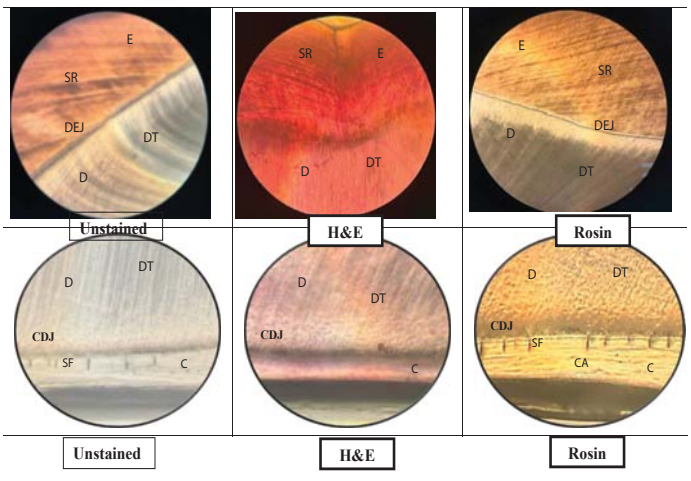


Figure 4: Photomicrograph of non-demineralized ground sections under Phase Contrast Microscope(10x) showing- Enamel(E); Striae of Retzius (SR); DEJ; Dentin(D); Dentin tubules (DT); Cementum(C); Cemental annulations (CA); Sharpey's fibres (SF); Cementocytes; Tome's Granular layer; Cemento-dentinal Junction (CDJ).

Table 1: Comparative Chart for Analysis of Unstained, H&E, & Rosin section under Compound Light Microscope.

Group	N(Sample)	LM(Mean)	Variance(One way ANOVA)	*p* value
Unstained	20	2.4	0.24	0.015
H & E	20	1.6	0.60	
Rosin	20	2.7	0.22	

Table 2: Comparative Chart for Analysis of Unstained, H&E, & Rosin section under Phase Contrast Microscope.

Group	N(Sample)	PC (Mean)	Variance(One way ANOVA)	*p* value
Unstained	20	2.68	0.22	0.001
H & E	20	1.94	0.46	
Rosin	20	2.89	0.099	

Discussion

Forensic odontology is an emerging branch of forensic medicine. The rising crime rates in our country have driven advancements in the field of forensic odontology. In recent years, the significance of dental identification has grown considerably. Thus, teeth become one of the most important pieces of evidence in forensic investigations. It plays a crucial role in age estimation, gender determination, dental DNA fingerprinting, tooth prints, bite mark analysis, and determining blood groups from dental pulp [6]. Age estimation is a vital domain of forensic science that plays a significant role in the identification process, particularly when information about the deceased is unavailable. Age estimation has commonly been performed using distinct tooth features suggested by Gustafson, such as attrition (A), secondary dentin deposition (S), apical migration of periodontal attachment (P), cementum apposition at the root apex (C), root resorption at the apex (R), and root dentin translucency (T).

Longitudinal ground tooth sections have numerous applications in oral histopathology because they allow a comprehensive view of most dental structures, such as enamel, dentin, and cementum [7]. Conventional methods for observing dental microstructures include preparation of undemineralized thin ground sections and decalcification, followed by staining with hematoxylin and eosin (H&E), basic/acidic fuchsin, toluidine blue, fast green, and silver staining reagents. However, these methods have limitations, including the two-dimensional view provided by ground sections that may not fully represent the intricate details of dental structures and the distortion caused by decalcification and histological processing. Additionally, conventional dyes tend to obscure critical structures in tooth sections, such as cross-striations in enamel rods, incremental lines in enamel, the enamel-dentinal junction, and incremental lines in dentin. Furthermore, laminar structures in cementum are not clearly visible in tooth sections stained with conventional dyes [8,9]. This highlights the need for high-quality, undemineralized tooth sections that are easy to handle, uniform in thickness, allow clear visualization of intact microstructures, and can be preserved for extended periods.

In the present study, we found that tooth structures were more clearly identifiable in Rosin-stained undemineralized

teeth compared to hematoxylin and eosin-stained teeth. Tissue sections were graded on a scale from 0 to 3 based on differentiation and contrast observed under both light and phase-contrast microscopy. Under light microscopy, the mean scores for rosin-stained, unstained, and H&E-stained sections were 2.7, 2.4, and 1.6, respectively (p = 0.015). Under phase-contrast microscopy, the mean scores were 2.89 for rosin-stained, 2.68 for unstained, and 1.94 for H&E-stained sections, with a p - value of 0.001. These statistically significant results demonstrate that the rosin staining method offers superior tissue differentiation and contrast compared to the other methods. This is likely because structures with limited mineralization and high organic content have a greater affinity for pigment, making these less-mineralized structures more visible and easily identifiable when stained with pigment-containing rosin [10]. Rosin staining was chosen because the refractive index of rosin is 1.527, which is slightly lower than the refractive index of enamel [11,12]. Additionally, the Abbe number can be increased to reduce dispersion. Since rosin contains a natural pigment, melted rosin exhibits good permeability [13,14]. When ground sections of teeth are soaked in melted rosin, partial pigmentation becomes visible in multiple tooth structures. The findings of this study align with the study conducted by Qizhong Qin, et al. [15], which demonstrated that rosin-stained ground sections of teeth facilitate the observation of microstructures within the teeth, particularly improving the textures of dental enamel, dentin, and cementum.

The present study showed that rosin staining is 30% more effective than unstained sections and 95% more effective than H&E-stained sections. Additionally, after staining with rosin, tooth structures exhibit a three-dimensional appearance, becoming more transparent and less prone to decolorization [16,17]. Figures 5,6 provides a comprehensive graphical comparison of rosin-stained, unstained, and H&E-stained sections. Graph 1 illustrates the ability of rosin staining to clearly delineate the incremental lines of enamel, Graph 2 demonstrates enhanced visualization of dentin translucency, and Graph 3 highlights the improved detection of incremental

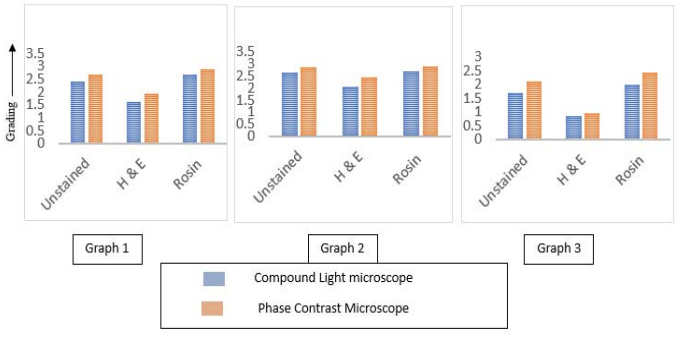


Figure 5: To compare Effectiveness of Rosin, H & E and unstained section for identifying Graph 1-incremental lines of Enamel; Graph 2- Dentin translucency; Graph 3- Incremental lines of cementum; by using Light microscope and Phase contrast microscope.

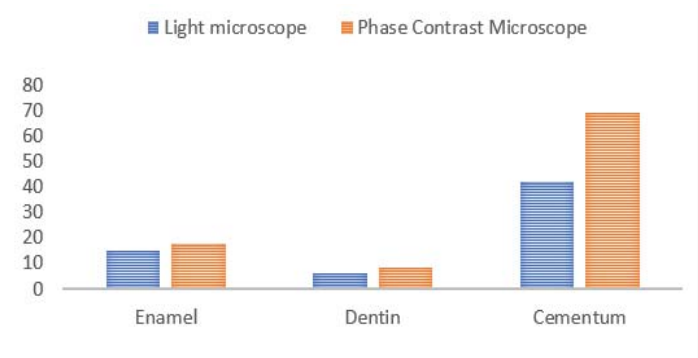


Figure 6: To compare the effectiveness of the light microscope and phase contrast microscope for identifying incremental lines of dental hard tissue.

lines of cementum. These observations, made under both light and phase-contrast microscopy, underscore the superior efficacy of rosin staining in identifying key histological features.

In this research, longitudinal sections were used to evaluate dental microstructures using a compound light microscope and a phase contrast microscope. Pundir, et al. [18] and Pradeep L, et al. [5] estimated age using the total cemental annulations method under phase contrast microscopy and found it to be much better and effective than light microscopy for evaluating the cemental lines. Rosin reduces background noise and light scattering, making less mineralized structures, such as incremental lines in enamel, dentin, and cementum, more pronounced under a phase contrast microscope. This suggests that Rosin-stained sections combined with phase contrast microscopy could be a more accurate method for age estimation.

One of the limitations of using rosin stain is its mechanical properties. It has a fast-setting time and forms a hard layer upon drying, which must be removed using xylene, adding an extra step to the process. Further research is needed to evaluate the grade of stained sections and the duration of the staining effect, as these variables were not assessed in the present study.

Conclusion

Rosin-stained ground sections, when examined under phase contrast microscopy, enhance the visibility and structural details of teeth. This method provides a more reliable approach for age estimation. These techniques hold substantial value in histopathology teaching, research, and forensic investigations, offering high accuracy and improved visualization.

Author's contribution

Dr. Kanika Dang– Conceptualization, Methodology, Writing – original draft

Dr. Mandakini Mandale – Final approval of the manuscript, Formal analysis.

Dr. Jayanti Humbe – Writing – review & editing.

Dr. Vaishali Nandkhedkar –Validation, Project administration,

Dr. Savita Wagh – Writing – review & editing.

Dr. Monika Kajalkar –Supervision.

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