Peels as an alternative to ground sections – An in vitro microscopic study

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Abstract

Background: The routine approach to delve into the organization of mineralized and non-mineralized structures of teeth is by studying whole tooth or slices of it by making thin section which requires laborious grinding or employing specialized equipment and also leads to specimen wastage. Peels hitherto utilized for fossil studies hold promise in overcoming the aforesaid shortcomings. Although the acetate peel technique has been modified for the study of tooth structure, the field remains largely unexplored.

Aim: The current study was taken up to explore the usefulness of cellulose acetate peels in reproducing microscopic structures of teeth as seen in routine ground sections and further if they could supplement or replace the same.

Materials and Methods: Extracted human teeth were embedded in plaster blocks in longitudinal and transverse orientation, ground and polished with silicon carbide paper. Following etching, washing and drying, the polished surface was wet with acetone and precut cellulose acetate film was placed over it and allowed to dry. As the acetate polymer dissolved in acetone and subsequently re-polymerized after setting into the micro reliefs produced by tooth etching, it registered the microscopic tooth details on its surface. The peels were mounted and secured on a glass slide and subjected to routine light and phase contrast microscopy for observing captured details of the tooth structure.

Results and Conclusion: Acetate peels successfully reproduced most of the microscopic tooth details which were better than those observed in ground tooth sections. Hence, this technique could be considered as a quick, durable and inexpensive alternative or supplement to routine thin ground sections of dental hard tissues.

Keywords: Acetate peels, dental hard tissue, ground sections, microscopic structures of tooth

INTRODUCTION

Routine sectioning of dental hard tissue employs various methods like dried ground sections prepared manually, sectioning by hard tissue microtome or decalcification followed by routine processing.¹ These slices are then subjected to various microscopy techniques such as light, polarizing and electron microscopy to elucidate their structural details.² Routine techniques usually lend a

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two-dimensional view of the microscopic structures, are labor intensive procedures, time consuming or need specialized equipment and pose a limit on the number of sections that can be prepared, invariably with a prior selection of good sections. They further may lead to sample breakage or wastage.\textsuperscript{3,4}

Peel making technique was pioneered by palaeobotanists (Walton 1928, Stewart and Taylor 1965) for intricate fossil study in plants and further developed by petrologists and paleontologists to study the structure of carbonate rocks and fossils, using an array of materials.\textsuperscript{3‑7}

A peel is a replica of an acid-etched surface made on a film.\textsuperscript{8} Cellulose acetate rapid peel procedures were introduced by Joy et al. followed by Galtier and Phillips and eventually modified for, depending on the nature of substrate including tooth.\textsuperscript{3,8,9} Peel technique involves dis-embedding a layer of any tissue by means of removal of the mineral matrix with acids and re-embedding the exposed cellular structures in clear acetate peel.\textsuperscript{3,8,9} Studies have shown the efficacy of the said acetate method in yielding a large number of sequential replicas from an undecalcified tooth.\textsuperscript{3,8,9} However, its role in reproducing structural details of hard tissues of a tooth is less explored. Thus, the objective of the present \textit{in-vitro} study was to explore the applicability of the acetate peel technique in reproducing microscopic tooth details as visualized in ground sections of teeth.

\textbf{MATERIALS AND METHODS}

Noncarious and unrestored extracted human teeth (n=25) with good morphology unaffected by attrition, abrasion, erosion or structural anomalies were collected and included in the study. The peel technique developed by Füsun \textit{et al.}, 2005, for studying dental hard tissues was employed with few modifications. The teeth were embedded in dental plaster blocks (Ca (SO4).1/2H2O, Neelkanth Minechem, Rajasthan) in longitudinal orientation in a few blocks and in cross-sectional orientation in some. The block was ground and wet polished successively using three grades of silicon carbide paper of 600, 800 and 1000 grit size (coarse, medium and fine), respectively (3M ESPE, USA) until the dental surface to be peeled was exposed and a flat surface was achieved. A smooth level surface helps avoid shallow puddles of acetone. It was further polished on a glass slab with water to get rid of any scratches that may have occurred due to grinding. The polished surface was washed and etched with 37\% orthophosphoric acid (Scotchbond; 3M ESPE; USA) for 2 min (time was extended to achieve greater structural detail), further washed in distilled water and left to dry. To make stained peels, polished surfaces of few teeth were dipped face down in Alizarin Red S staining solution (Nile chemicals, Maharashtra) for 5 min, washed and left to dry. Commercial grade acetone solvent (Rankem; Ranbaxy Chemicals Ltd; India) was squirted over the entire etched and/or stained surfaces of the teeth using a glass pipette and a piece of pre-cut transparent cellulose acetate strip of 0.003 inches thickness (Grafix Acetate; Pennsylvania, USA) was aligned with the block and immediately lowered on the acetone drenched surface, in a manner so as to avoid air bubbles and without applying any finger pressure. The acetate film was initially affirmed for applicability by performing the fingerprint/palm print test so as to verify if it could produce a palm print when the palm is wetted with acetone and pressed on it. As the acetone evaporated, the wet acetate sheet settled into the micro reliefs produced by prior etching procedure, to register a detailed impression of the tooth surface. As the peel dried up in about 30 min, it was gently peeled away from a corner without applying pressure. Surplus peel material at the sides was trimmed and the peel was immediately wet mounted using distilled water (temporarily) on a glass slide and covered with a coverslip. Further, they were examined in transmitted and incident light, in phase contrast under different magnifications of the microscope and microphotographs were captured using a dedicated camera mounted on a microscope (Olympus BX 41, ProgResC® 3 camera, Genoptix; Germany). Most of the peels showed a three-dimensional (3D) image of the registered tooth surface on microscopic examination, indicating the accuracy in capturing the relief areas/points. Peels both from crown and root areas were studied. Surface peels of unetched tooth surfaces were also made and studied. The peels later were preserved by drying and mounting them between two glass slides (25 mm × 75 mm) and securing the edges with adhesive tape [Figure 1].

\textbf{RESULTS}

The peels when studied in various combinations of light and magnification were able to reproduce almost all the gross and fine structural details of enamel, dentine and cementum. Our observation with numerous peels revealed that details of enamel and dentin were more appreciable and precise than that of cementum, probably owing to lesser inorganic content in cementum. Other structures such as Tome’s granular layer, interglobular dentin, primary and secondary dentin could also be discerned. Figures 2-4 depict some of the microscopic images captured in the acetate peels in comparison to photomicrographs taken from routine ground sections from the departmental archives. Table 1 draws a comparison between ground sections and acetate peels with regard to microscopic features of the mineralized tissues of the tooth.
**DISCUSSION**

The imprint of etched surfaces on transparent plastic is a rapid and simple method to study anatomical details of tissues. Acetate replicas can be made by dry peel technique or by making peel sheets in the laboratory by pouring solutions like ethyl cellulose in trichloroethylene onto glass plates. Commercially available preformed acetate sheets are however currently preferred due to uniformity in thickness and ease of mounting. Acetate peels in particular have proven to be of value in studying carbonate cements and textural criteria. Cellulose acetate is easy to use,

![Figure 1: Photographs of the modified acetate peel technique: (a) Commercially available Acetate sheets, (b) Palm print test to ascertain the right acetate sheet, (c) Grinding tooth block embedded in plaster for a flat surface, (d) Acid etching tooth surface to produce microrelief, (e) Flooding surface of the tooth with acetone, (f) Immediate placement of acetate strip on acetone drenched tooth surface, (g) Swift removal of acetate strip from tooth after drying, (h) and (i) Final unstained and stained acetate peels in longitudinal and cross-sections of teeth](image)

![Figure 2: Comparative photomicrographs of structures seen in enamel and dentin on acetate peels (a-d) and on ground sections (e-h) (a and e: Enamel rods, dentinal tubules, dentino enamel junction), (b and f: Enamel tufts), (c and g: Enamel spindles), (d and h: Cemento enamel junction) (Magnifications used: ×100 and ×200 in routine light microscopy, a-phase contrast, ×100)](image)

| Microscopic structures | GS | Acetate peels |
|-----------------------|----|--------------|
| Enamel                | Enamel rods, tufts, lamellae, spindles, incremental lines, Hunter Schreger bands, gnarled enamel are structures commonly discernible | All microscopic structures visualized in GS discerned. Renders superior visualization of differential arrangement of enamel rod patterns |
| Dentin               | Dentinal tubules, dentin enamel junction, different types of dentin s predentin, primary dentin, secondary dentin, interglobular dentin and Tome’s granular layer are commonly viewed structures | Surface structures like perikymata and aprismatic enamel can be viewed. Most of the dentinal structures could be fairly discerned. Dentinal tubules with remnants could be captured giving a 3-D view of the tissue |
| Cementum            | CEJ, cement-dentinal junction, cellular and acellular cementum, incremental lines are structures commonly seen | Microscopic structures in cementum were challenging to view. CEJ was the structure best captured |

GSs: Ground sections, CEJ: Cementoenamel junction

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**Table 1: Comparison between ground sections and acetate peels in defining the different microscopic features of mineralised tooth tissues**
Rath and Raghunath: Can peels replace ground sections in a routine study of the microscopic structure of dental hard tissues?

We performed a preliminary study using acetate peel technique in extracted teeth and registered tooth structures as intricately as fingerprints. The principle of action upon which the technique is based on is an exchange process by which inorganic tooth material is exchanged for synthetic resin. The acetate ester of cellulose initially solubilized by acetone (owing to a similar solubility coefficient), then hardens by in situ polymerization of the resin that is readily absorbed by capillary attraction into the created etch pits as the acetone evaporates, forming resin tags. Hence, once peeled off, the acetate strip would have registered tooth structure intricately, in fact, close to obtaining a replica of the tooth sections. Acid in the etchant reacts with the hydroxyapatite of tooth structure, but not with the organic remains, so the mineral content is etched away producing a microrelief on the surface of the tooth. Reports claim that etching may be the single most important step in preparing good quality peels. As the acetate strip is quickly rolled with flexed tension on the tooth surface, the acetone, an organic solvent, partially dissolves the lower surface of the acetate sheet, converting it to a liquid that flows in around the intricacies of the tooth structure and solidifies embedding the cellular details within it. Therefore, using too thin sheet can result in peel wrinkling and insufficient acetone may result in poor reproduction of detail. We employed Füsun et al’s technique incorporating few modifications (plaster block for embedding tooth and silicon carbide papers of differing grits for grinding and polishing) as per the purpose of our study, to yield an economic and feasible method.

Most of the structural tooth details were captured, simulating those seen in ground tooth sections. Even surface structures such as perikymata and aprismatic enamel, 3D appearance of dentinal tubules with odontoblastic processes jutting out and enamel rod patterns were discerned in peels, apart from registering all the routinely seen structures under routine ground tooth section.

Figure 3: Photomicrographs of enamel on acetate peels: (a and b) Enamel rods in longitudinal section, (c) Stained enamel prisms (Alizarin Red S), (d) Transverse section depicting enamel rod orientation like fish scale pattern, (e and f) Hunter-Schreger bands, (g) alternating longitudinal and transverse prisms, (h) Deviating gnarled appearance of prisms at cusp tips, (i) Aprismatic enamel, (j) Perikymata (Magnifications used: ×100, ×200 and × 400 in routine light microscopy and phase contrast)

Figure 4: Photomicrographs of dentin and cementum on acetate peels: (a and b) Dentinal tubules with their openings, (c) Few odontoblastic processes from tubules in cross-section, (d) Y shaped dentinal tubules, (e) Incremental lines of cementum, (f) Cemento-enamel junction (overlapping type) (magnifications used: ×100, ×200 and × 400 in light microscopy and phase contrast)
Table 2: Merits and demerits of Ground sections and Acetate peel technique

| Advantages and pitfalls | GS | Acetate peels |
|-------------------------|---------|---------------|
| Microscopic visualization of hard tissues of tooth | Gold standard and routine procedure | Employable as an alternate technique to GS |
| Cost and labor | Cheaper as compared to hard tissue microtomy but manually tedious | Cheaper as compared to hard tissue microtomy, but costlier than GS. Less labour intensive |
| Stability and storage | Stable and can be stored | Peels are stable and can be stored dry |
| Rapidity | Time consuming | Rapid and time saving |
| Serial sections | Not possible | Possible |
| Surface structure visualization and 3-D view of tooth topography | Not possible | Possible |
| Applicability with different microscopy and photography techniques | Yes | Yes |
| Routine practical work curricula in oral histology for undergraduates | Can be considered due to simple technique and low cost | Hard to be considered owing to the high cost and technique sensitivity |
| Tooth specimen wastage | Yes | No |
| Injuries | Possibly to fingers while manual grinding | No |
| Inflammable constituents | No | Yes (due to acetone) |
| Curling of sections | Not encountered | May be a problem and has to be avoided |
| Simulating SEM and TEM sections | Not possible | The enamel rod alignment in different patterns, surface structures and odontoblastic process within dentinal tubules could be appreciated. So can simulate SEM and TEM sections |

GS: Ground section, SEM: Scanning electron microscope. TEM: Transmission electron microscope

only one previous study employing acetate peel technique application to visualize microscopic structures of teeth, the authors studied gross and fine structures in tooth and found it to be a useful supplement to thin sections. Their pioneering study laid down guidelines for making dental acetate peels for the microscopic examination which was successfully adopted (albeit with modifications depending on working environmental conditions) in the current study to exploit the technique more intensively in eliciting most of the tooth structural details. We explored both longitudinal and transverse tooth sections at different levels in crown and root, using routine light microscopy and phase contrast microscopy. We studied few peels mounted in distilled water unlike permanent mountants used for ground sections.

Other investigators have utilized dental acetate peels in the field of forensic odontology to study the uniqueness of enamel rod end patterns or tooth prints (ameloglyphics) in personal identification by combining the technique with automated bimetrics. Its practical application in differentiating dental hard tissues of humans from other species or salvaging precious tooth samples in forensic scenarios by making peels of the same until further investigations can be carried out needs to be explored.

Few advantages of the method include first observation of specimen characteristics, routine use with a binocular microscope just like study of any thin section and possibility of obtaining very close serial sections which would prove helpful in 3D reconstruction of tooth. A quick smoothening of the surface with fine-grit carbide paper is the only requisite for repetition of the process. As many as 15–20 peels may be obtained from 1 mm thickness of tooth. Peels provide information on the content and permit rapid selection of the best specimen. Peel techniques are straightforward methods, practical and easy to execute. They are suited for high magnification studies. Peels are amenable to photography, can be placed in photographic enlargers for producing drawings and used with grids for quantitative analysis too. They make for inexpensive “copies” which can be utilized in projection onto large screens, microscopic examination and in some instances, in preparation of photographic illustrations. They can be readily filed for ready reference in a small storage space, are practically indestructible in routine work and last indefinitely. Table 2 enumerates the advantages and pitfalls of acetate peels in comparison to ground sections of teeth.

Few precautions need to be exercised with this technique like storing the acetone in a well-ventilated area due to its toxic and inflammable nature. Acetone may evaporate from the specimen surface before the acetate sheet adheres resulting in bubbles if there is excessive high temperature in the working environment. The peels are also inflammable and may be stored in labeled envelopes in a dry place. Few disadvantages however remain like acetate films are susceptible to contamination and have a tendency to curl up. Minerals cannot be identified by optical properties like birefringence with peels (inability to visualize optical properties of crystals).

CONCLUSION

The acetate peel technique could be a useful alternative or supplement to ground tooth sections and can be further
explored to assess its adaptability to various research and field-use purposes. In the present study, the valuable anatomical information obtained from peels can be applied further to development of other approaches for the study of normal and pathological human as well as nonhuman dental hard tissues.

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Conflicts of interest
There are no conflicts of interest.

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