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Acetate peel technique: a rapid way of preparing sequential surface replicas of dental hard tissues for microscopic examination

Alkaya Füsun [a], Özer Füsun [b], Belli Sema [b], Karakaya Solen [b,*]

[a] *Department of Geological Engineering, Faculty of Engineering and Architecture, University of Selcuk, Campus, Konya, Turkey*
[b] *Department of Operative Dentistry, Faculty of Dentistry, University of Selcuk, Campus, Konya, Turkey*

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Method;
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Summery

Objective: In order to study dental structures in three-dimensional views, sequential microscopic sections are needed. Routine thin-sectioning needs equipment, it is time-consuming, expensive and cause sample wastage during sectioning. The acetate peel technique used for microscopic examinations of carbonate rocks and fossils was modified and successfully adapted for teeth.
Design: A human tooth was embedded in resin block; the surface to be peeled was ground and polished with carborundum powder on a glass plate using water. The polished surface was etched, washed, and dried. The dry surface was flooded with acetone and a piece of acetate film was placed over it. As the acetone evaporated, the film settled down to take the shape of micro relief produced by etching. The film dried in 10—15 min. It was pulled off the surface and mounted between two glass slides for examination under microscope.
Results: This technique enabled making numerous sequential surface replicas (peels) from a tooth, especially from fully mineralised enamel without routine decalcifying, dehydrating, sawing, and mounting processes.
Conclusions: The results obtained with the stained peels, showed that this technique is an efficient way to study gross and fine structures of etched dental hard tissues surfaces and restoration-cavity marginal integrity with three-dimensional detail.

Introduction

Three-dimensional investigations of dental hard tissues need a large number of thin serial sections. Without decalcifying processes only a small number

* Corresponding author. Tel.: +90 332 223 12 47; fax: +90 332 241 00 62.
E-mail addresses: karakaya@selcuk.edu.tr, solen885@hotmail.com (K. Solen).

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of whole sections can be made from a single tooth. Thin-sectioning techniques for undecalcified or partially decalcified teeth were introduced by previous workers.[1,2] Decalcification or partial decalcification of enamel may solve this problem, however these procedures are time-consuming, need special equipment, and considerable artifacts are introduced. The acetate peel technique described here provides the researchers with a rapid way of preparing a large number of sequential, replicas from an undecalcified, but etched tooth surface. The replica is easy to make, inexpensive, does not need thin-sectioning devices, and perfectly serves for the purpose.

A peel, simply, is a replica of an acid-etched surface made on acetate film. The peel-making technique was first developed by palaeobotanists[3] to study the cellular structures of fossil plants. Following the pioneering studies of palaeobotanists, carbonate petrologists and palaeontologists have developed similar techniques to study both the texture and structure of carbonate rocks and fossils.[4–6] Depending on the nature of substrate and purpose of the study, peels can be prepared in various ways. In the present study the cellulose acetate peel procedure was found to be the most efficient for making dental peels. Since the mineralogical composition of dental hard tissues is different from those of fossil plants and carbonate rocks the procedure was modified.

Materials and method

Peel material and solvents

Peel sheets can be made in the laboratory,[7,8] however they are fragile, non-uniform in thickness, and time-consuming to prepare. It is more convenient to use commercially produced acetate sheets. They are available in sheets and rolls in a wide range of thickness. Acetate sheets are soluble in methyl acetate, ethyl acetate, ethyl lactate, diacetone alcohol, and tetrachloroethane.[6]

In the present study 0.1 mm (or less) thick commercially produced cellulose acetate film and commercial grade acetone solvent were used. In order to choose the right type acetate film for acetone solvent the finger is wetted with acetone and pressed on to the film, if a fingerprint is produced then it is the right type acetate.

Surface preparation

A human mandibular right second molar tooth was embedded in a block of epoxy resin (Epofix, Struers, A/S), with the occlusal side close to the block surface.

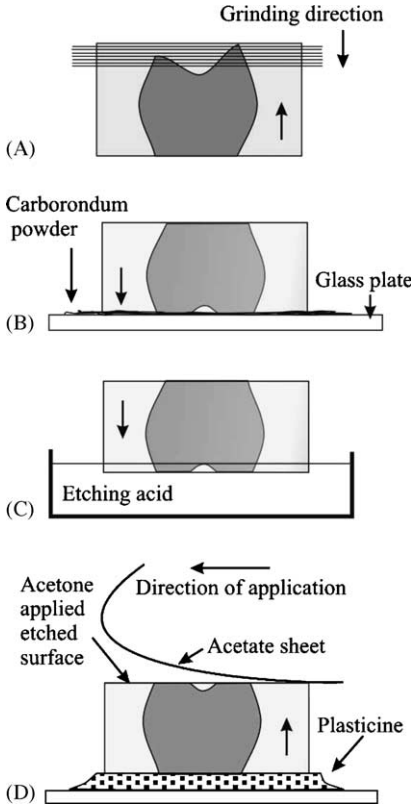

Figure 1 Peeling procedure of the tooth.

For convenience the root was cut (Fig. 1A). The resin block was ground with coarse grade carborundum powder using water on a glass plate until the dental surface to be peeled was exposed (Fig. 1B).

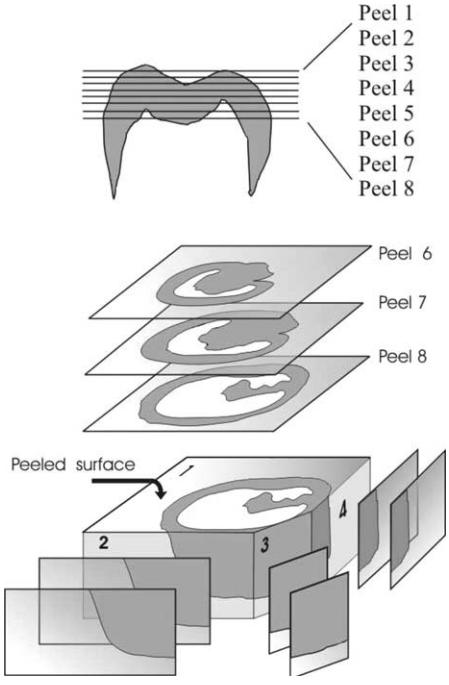

Figure 2 Schematic block diagram showing peeling directions.

The surface was thoroughly washed and polished with 800 grade carborundum powder on a separate glass plate. The polished surface must be flat and perfectly smooth. If the glass plate is not changed during final polishing relicts of coarse grade powder may cause scratches on the surface. For greater details, further smoothing by polishing with 1 μm Aloxite or diamond abrasive is recommended.[6] If carborundum powder is not available the cut surface can be wet-polished using wet silicon carbide papers and diamond pastes of decreasing abrasiveness down to 0.25 μm.

Etching the polished surface

The polished surface was thoroughly washed with tap water then it was etched holding face downwards (Fig. 1C) and agitating slightly in orthophosphoric acid (37%) for a period of 1—2 min. The etched surface was washed in distilled water and left to dry.

The etching time can be shortened or extended by researchers depending on the purpose of their study and on the degree of detail required. The etched surface must not be touched, as the delicate relief may be damaged.

Staining (if required)

Chemical staining involves a reaction which produces a colored precipitate on a specific mineral surface and therefore makes that mineral more easily recognised. To prepare stained peels, after the etching stage the surface is immersed in the staining solution, staining takes about 2—3 min (some experimentation is recommended for different

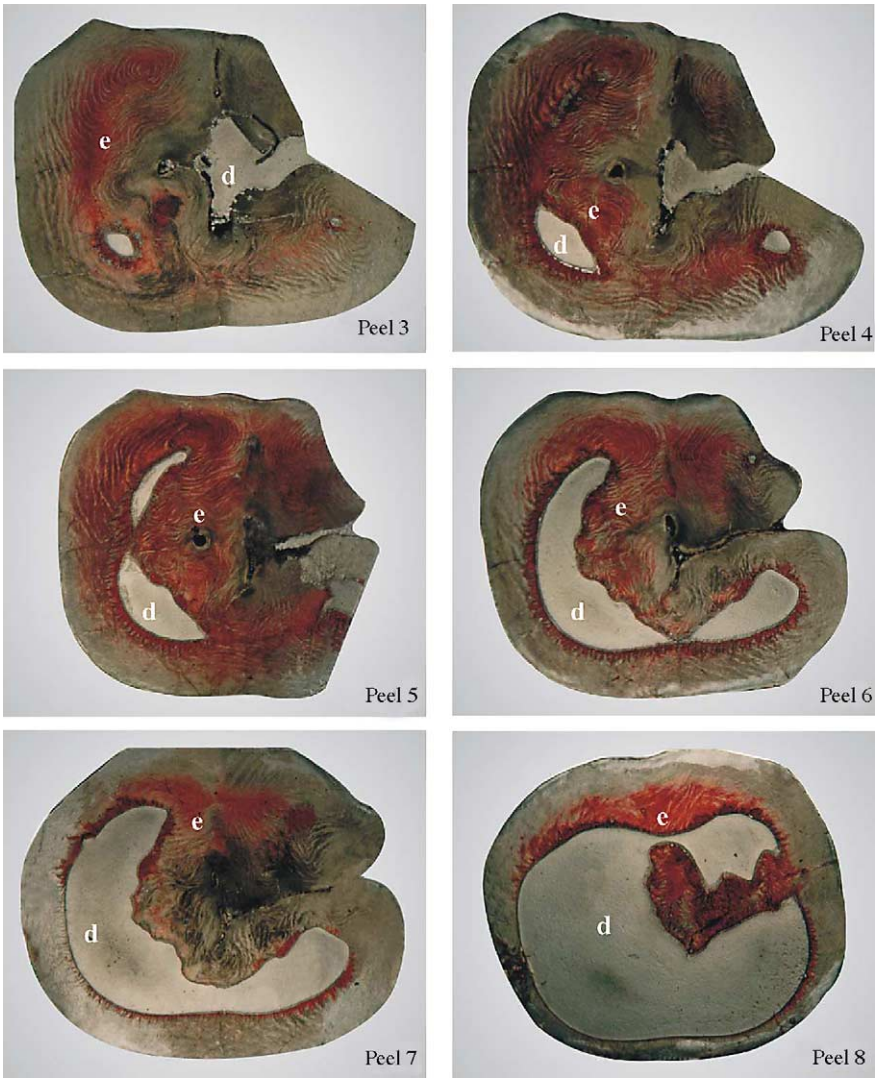

Figure 3 Serial transverse sections of a human mandibular right second molar tooth. Note the alternating bands of transversely (red-stained) and longitudinally sectioned prisms (Hunter—Schreger bands): d, dentin; e, enamel.

staining solutions). The stained surface is washed and left to dry.

In our work we used an organic stain, Alizarin red S, which is specific for calcite ($CaCO_3$). What kind of chemical reactions took place is still unknown to us but it worked perfectly to bring out patterns of decussating enamel prisms (sometimes used synonymously with Hunter—Schreger bands).

Peeling and mounting

After etching (and staining) the resin block was fixed with plasticine (or modeling clay) on a glass plate so that the etched surface was oriented face-up and horizontal. The etched surface was flooded with acetone and a piece of pre-cut acetate strip was placed over it by bending the acetate into the acetone-drenched surface to drive-off the air bubbles (Fig. 1D). As the acetone evaporated the film settled down along the micro relief produced by etching and took a detailed impression of the acid-etched surface (the film adheres to the surface without outside pressure, fingers should not be pressed on the surface). The film dried about in 20—30 min. It was removed by gently peeling away from one corner and the excess film was trimmed from around the peel. The peel was placed between two glass slides and the edges were bound with adhesive tape. The section was ready for examination under microscope.

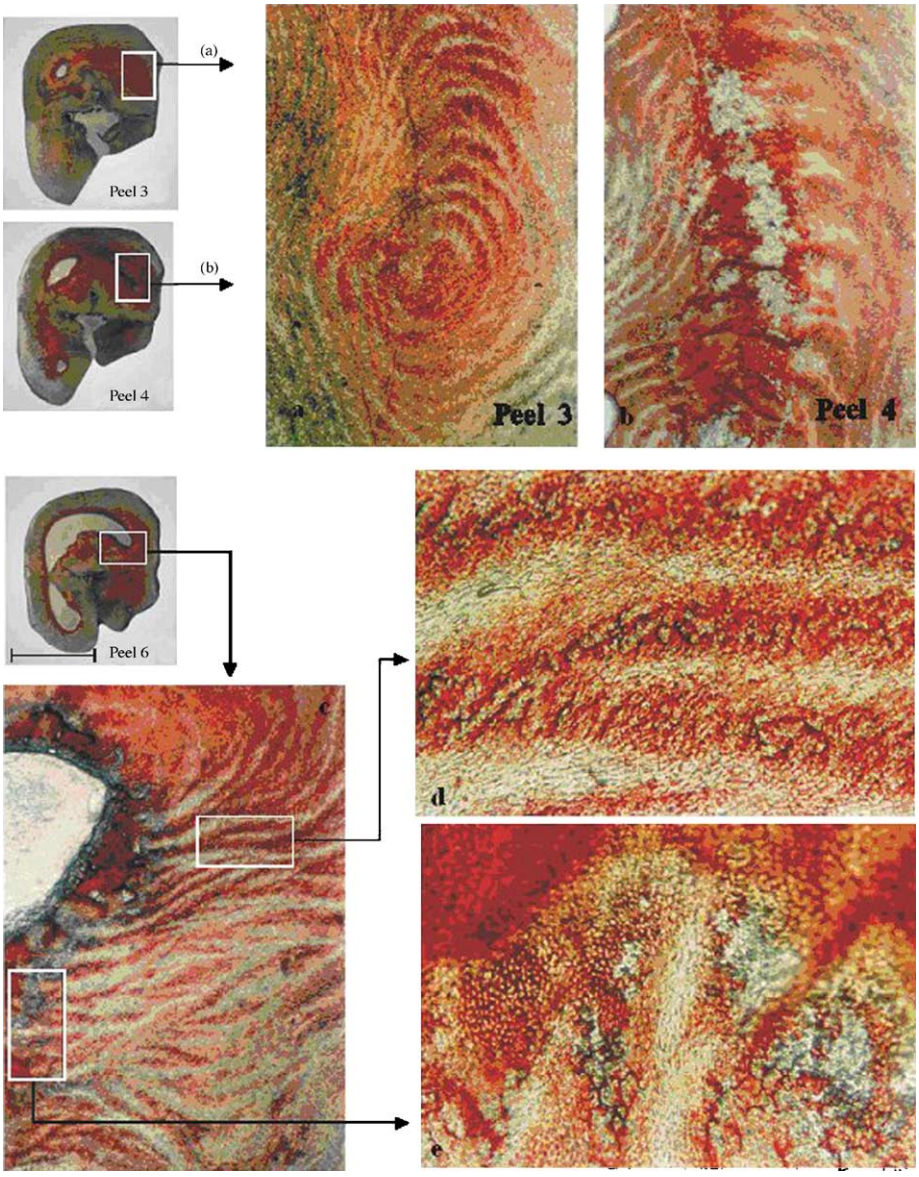

Figure 4 (a, b) Two successive transverse surface preparations of mesiobuccal cusp (peels 3, 4). *Note*: Hunter—Schreger bands spiraling around the apex of the cusp; (c—e) details of the marked areas in peel 6 (bar = 0.5 cm).

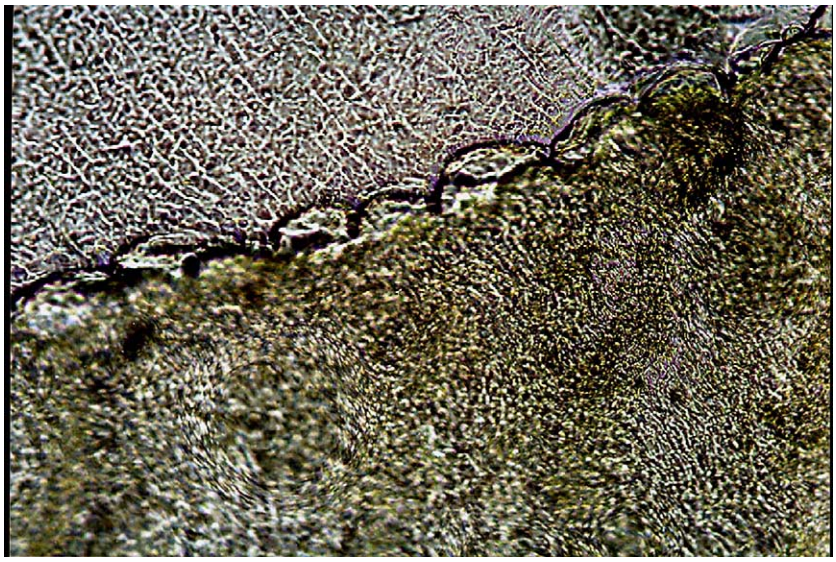

Figure 5 Dentino-enamel junction with scalloping structure (original magnification 20×).

Preparing sets of serially taken peels

After each peel, by re-grinding the surfaces, a further set of serially taken peels was produced (Figs. 2 and 3). This provided the topography of the tooth. One of the advantages of the method was that during the serial peeling the direction of peeling could be changed to give a different three-dimensional visualisation of the examined structures (Fig. 2).

Examination of the peel under microscope

The peels were examined by transmitted and incident lights. To increase contrast, oblique incident and transmitted light combinations were used for viewing the relief to gain a three-dimensional effect. The micro relief was also viewed by slightly changing the plane of focus. When parallel light (without substage condenser) was used to illuminate the section, as in thin sections, fringes occurred, therefore experiments needed to be done with and without the substage condenser in place. When taking the photographs blue filter was used on the illuminator.

Advantages of the method

Using the acetate peel technique we were able to prepare numerous sequential surface replicas of an undecalcified tooth. In Figs. 3—6 some

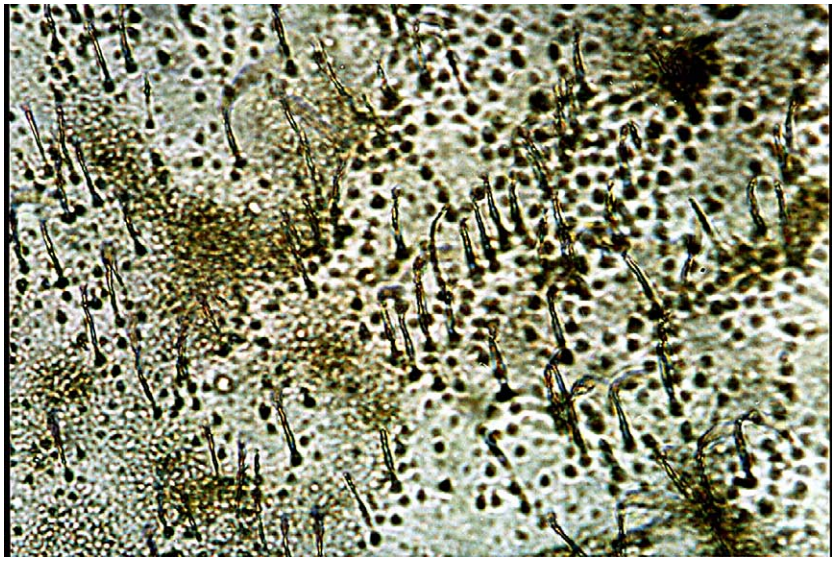

Figure 6 Dentinal tubule traces in the replicas of acid applied dentin surface (original magnification 20×).

examples of microscopic images are given. Advantages of the method can be summarised as follows:

1. Peeling is a simple, inexpensive, and rapid way of making replicas of dental hard tissue surfaces.
2. A set of serially taken peels can easily be produced by re-grinding surfaces after each peel. This provides a view of the topography of tooth structure.
3. Immediately after peeling, the peel can be examined under microscope with incident or transmitted light or with combinations of them.
4. A peel gives an overall view of a surface, if it is examined before next grinding, it can be used as a guide for further detailed work (i.e., if a specific area is marked during the microscopic examination of a peel, the remaining peeled surface can be used for SEM investigation or thin-section making).
5. The direction of peeling can be changed at any stage for three-dimensional visualisation of examined structures.
6. Using different stains two or more differently stained peels can be taken from the same surface.
7. The etching time can be extended by re-etching the same surface after each peel and each time either the same or different etching solutions can be used. Comparison of these peels enables detection of different mineral species and their distribution.
8. Peels are stable and can be stored.

Conclusions

The acetate peel technique described here is an easy, rapid, and inexpensive way of preparing sequential replicas of dental hard tissue surfaces for microscopic examinations. Using the technique we prepared a large number of dental peels for various purposes. The Peels revealed not only gross structures (i.e., pattern of Hunter—Schreger bands and Retzius lines) but fine structures (i.e., cross striation of enamel prisms) as well. It was possible to apply the technique to the investigations of restoration-cavity marginal integrity. Peels were found to be useful supplements to thin sections.

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