OBSERVATIONS FROM SHADOWED COLLODION REPLICAS OF TEETH WITH AMALGAM RESTORATIONS

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THE USE OF conventional or increased handpiece operating speeds for the shaping of cavities in human teeth results in vibration, temperature changes, and other physical factors within the calcified tooth tissue and its organic matrix. The airbrasive and ultrasonic methods of cutting tooth tissue appear to create similar forces. The effects of these trauma may or may not be significant to the longevity of the healthy functional tooth; this may depend somewhat on the severity of the forces involved.

The ultimate goal of this project is the study of the traumatic effects on both hard and soft tooth tissue which might develop when dental instruments are operated under various conditions. Although the actual development of observable changes in tooth tissue due to rotating instruments is conjecture, it seems likely that some alteration of the normal histologic pattern could occur.

Extracted teeth containing amalgam restorations were selected for study by the shadowed collodion replica technic in order, first, to establish a method of preparation which would allow rapid and accurate examination by the optical microscope, and second, to survey these specimens for any changes from normal which may have occurred. Shadow-casting for optical microscopy, using collodion as the substrate and aluminum as the metallic film,[25] and the general suggestions of Scott and Wyckoff[13, 14] for replica preparation were used as a basis for the development of the technic.

This paper presents in some detail a technic for preparing shadowed collodion replica specimens of sectioned teeth containing restorations, for the general examination of the hard tooth tissue with the optical microscope. Some observations of both normal and affected tooth tissue are presented.

REVIEW OF LITERATURE

Wolf[26] described an "adhesion relief method" for the study of tooth tissue in which celluloid impressions were taken of exposed surfaces. These impressions, which were removed with a sticky cellophane tape, were studied in the optical microscope with transmitted light, usually with oblique relief lighting.

The necessity of having extremely thin specimens in order for investigations to be carried on under the electron microscope[2] resulted in the use of surface replication. According to Zworykin,[28] Mahl formed oxide films on aluminum which produced a negative replica following the removal of the metal. This

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author also described collodion and vinyl lacquer replicating materials for negative replicas and a silver-collodion method for making positive replicas. Other replicating technics and materials have been described in the literature.[1, 4, 5, 11, 12] The topography of the replicas and their interpretation have received comment.[6-8] Richards and Thomassen[10] described the use of the polystyrene-silica replica for the electron microscopic examination of teeth. Gerould[3] also used this method.

Shadow-casting was introduced by Williams and Wyckoff[21] as a procedure for measuring heights of objects on replicas. Shadow-casting was also used as a method of increasing the contrast under the electron microscope, thus producing a three-dimensional, more detailed object.[9, 20, 22-24] It was evident that there was a definite place in optical microscopy for shadow-casting[25]; Scott and Wyckoff[13-15, 18] demonstrated some of its uses in dentistry. Metal shadowed replicas have provided excellent specimens of teeth for electron microscopy also, and numerous reports of these findings have been published.[16-19, 27]

TECHNIC OF SHADOWED REPLICA PREPARATION

From a group of freshly extracted teeth which had been stored in 70 per cent alcohol, an attempt was made to choose a posterior tooth with an amalgam restoration and the remaining tooth structure in reasonably sound condition. A close-up photograph was taken to permit future orientation of the replicas. The tooth was then immersed in 70 per cent alcohol.

1. *Sectioning.*—The tooth was mounted in impression plaster in a square brass block which had a tapered hole through it, approximately ⅜ inch in diameter. The crown was so exposed that the proper sectioning plane was secured. The brass block was screwed tightly in place on a sliding carriage which passed into the path of a disk attached to a lathe engine. The movement of the carriage into the disk and the thickness of the section produced was easily controlled. An 0.013 inch thick aluminum oxide disk* was run at high lathe speed with a constant stream of water directed from each side to the cutting edge. Each section, 1 to 2 mm. thick, was numbered in pencil on the undesired side and returned to its respective bottle.

2. *Polishing.*—Each section was polished on No. 3/0 metallographic polishing paper, then rinsed off; polished on No. 4/0 paper, rinsed off; and finally polished on wet green felt with an aqueous solution of white rouge. The section was thoroughly washed and returned to the specimen bottle. The use of the green felt resulted in some greater selective polishing of the dentin and periphery, and for this reason, may be undesirable for certain studies. It does, however, remove the noticeable scratches remaining from the No. 4/0 paper.

3. *Etching.*—The polished specimen was removed from the 70 per cent alcohol and blotted dry. It was then immersed in 0.05 N HCl for 1 minute, the acid being constantly agitated. At the end of 1 minute, the section was removed from the acid, rinsed in distilled water, blotted dry, and the surface to be replicated dehydrated with 3 or 4 drops of 90 per cent alcohol for 2 minutes, then absolute alcohol for 2 minutes. The sections were then blotted dry and allowed to stand for a few minutes before proceeding. The etching and replication were done in a semi-dustproof enclosure.

*XA-1803-P-RR-5 rubber bonded aluminum oxide wheel, 3-inch diameter with ½ inch arbor hole, 0.013 inch thickness. Allison Co., Bridgeport 8, Conn.

4. *Replication.*—With the surface of the specimen dry, a clear collodion solution was applied with a glass rod. This solution was composed of: 2 parts—flexible collodion, 1 part—amyl acetate, and 1 part—ether. The first clear coat was allowed to dry thoroughly for 15 minutes. At the end of this time, a second coat of the collodion mixture was similarly applied, only to 1 ounce of this solution a small crystal of crystal violet had been added. This stain facilitated the handling of the replica by making it more visible. The second collodion coat was allowed to dry, then the specimen was immersed in cool water. This water immersion appeared to have no detrimental effect on the collodion, and the stripping or peeling of the replica from the tooth section was greatly facilitated. The removal was started by cutting between the replica and tooth with a scalpel, then with microforceps the freed edge was easily grasped.

   The replica was then floated impression side up on a drop of water on a microscope slide which had previously been coated with a thin film of albumin-glycerin fixative. When the slide was placed in an oven at 55° C. for 10 minutes, the replica flattened and became rather firmly attached to the glass.

5. *Shadowing.*—A combination of a mechanical and diffusion pump, capable of pumping down to approximately 5×10^{-5} mm. of mercury, was used to evacuate a large bell jar. An electrode, whose height was adjusted to 16 mm. above the shadowing table, carried a tungsten filament wound to form a basket. Into the basket was placed 1.5 cm. of 24-gauge aluminum wire which was to be vaporized in the high vacuum. The replica was positioned 16 cm. away from the base of the electrode. This resulted in a shadow being cast the same length as the height of the object causing the shadow.

6. *Mounting.*—After the shadow-casting with aluminum, the slides were permanently mounted. A cover slip, 22×40 mm., No. 1 thinness, covered the 2 to 3 replicas on each slide. The mounting medium was Harleco synthetic resin 60 per cent in toluene. Weights were placed on the cover slips and the slide placed in an oven for 18 to 24 hours at 55° C.

Although the sections in this report were prepared in the above manner, the use of a cold-cured resin, Ward's Bioplastic, as an embedding medium has since proved useful in supporting weakened tooth specimens, and as an aid in polishing without rounding the periphery of the specimen. After sectioning, the embedded specimens can be polished on 400 and 600 grit silicon carbide paper under a stream of water, then they can be finished on a metallographic polishing wheel with various cloths and abrasives.* This alternate method of polishing allows all work to be carried out in a wet field which provides a more desirable section.

OBSERVATIONS

Since the shadowed collodion replicas are actually negative impressions, the observations become strictly topographical, and the histologist must change his concept of interpretation when viewing the shadowed replica. With this method, shades of gray, produced by the angular shadowing of an irregular surface with metal, are viewed. The technic supplements the conventional histologic preparations, and presents another method of collecting information concerning the hard tooth tissues which may lead to a more complete understanding of their normal histology and pathology. The technic of specimen preparation is simplified, although some of the equipment, namely the vacuum unit, is rather specialized and costly.

*Microcloth (1577AB) with levigated alumina (1557 AB) and kitten-ear broadcloth (1598 S) with levigated shamva (1558 AB). Buehler, Ltd., Evanston, Ill.

The dentin spikes are observed in all sectioning planes, but are more easily demonstrated in the transverse sections (Figs. 1, 2, 3 and 12). There is a zone apparently clear of spikes adjacent to the dentinoenamel junction; this was also observed by Scott and Wyckoff.[15] Although this clear zone decreases in width, it follows around the base of the restorations (Fig. 3). The spikes become more numerous, up to a point, as one moves away from the dentinoenamel junction, then thin out and disappear as the pulp area is approached (Fig. 12). These spikes may be the result of the flow of collodion into the tubules, and since the tubules become smaller in diameter near the dentinoenamel junction, and as sclerosis may occur under caries and restorations, the collodion here may penetrate only a very short distance. The spikes may also result from the removal of some organic material from the tubules when the replica is stripped.

An enamel lamella-like structure (Fig. 4) extends from the dentinoenamel junction to the enamel surface. It is possible that this lamella is a crack produced by the forceps during extraction or during the polishing at the sectioned tooth. In order to alleviate this possibility for the most part, teeth to be extracted for this type of study may be removed with caution and the areas to be studied can be progressively cut, polished, replicated, then recut.[15] This would eliminate the handling of sections even 1.5 to 2.0 mm. in thickness. Embedding of the tooth in the cold-cure plastic would be of value here.

Fig. 5 shows a restoration which parallels enamel rods and Fig. 6 shows one where enamel is unsupported by dentin. Other sections, at both proximal and occlusal margins, have shown distinct undermining and subsequent cracking. It has also been demonstrated that where the dentin apparently is supporting the enamel, a crack has developed nevertheless. Still other sections have shown enamel, unsupported by dentin, with no apparent cracks.

Fig. 7 shows the base of an unfilled fissure which, from gross observation of the tooth section, appeared to have begun decalcification. The dark areas to the right and left of the fissure correspond to the opaque areas on the tooth section. On the replica, these dark areas have received a more even coating of metal; they are smoother then, and apparently were little affected by the etching after polishing. The enamel below the fissure appears coarse. In Fig. 8 the dentinoenamel is shown adjacent to the base of restoration. The enamel near the junction has similar dark areas with coarse enamel running through these. The corresponding areas on the tooth section were opaque.

The enamel immediately below the restoration in Fig. 9 has no rod structure and appears to have been infiltrated by the restorative material. Below this area in Fig. 9 and the enamel immediately adjacent to the restoration in Fig. 10 show again the apparently abnormal coarse arrangement of rods. The observation of this type of enamel adjacent to a restoration was a general finding. This appearance is probably derived from thin vertical projections of the replica, thus casting many shadows (clear area) separated only by a thin dark line (metal coated). The cause and significance of this irregular enamel present a challenging study.

Fig. 1. Fig. 2.

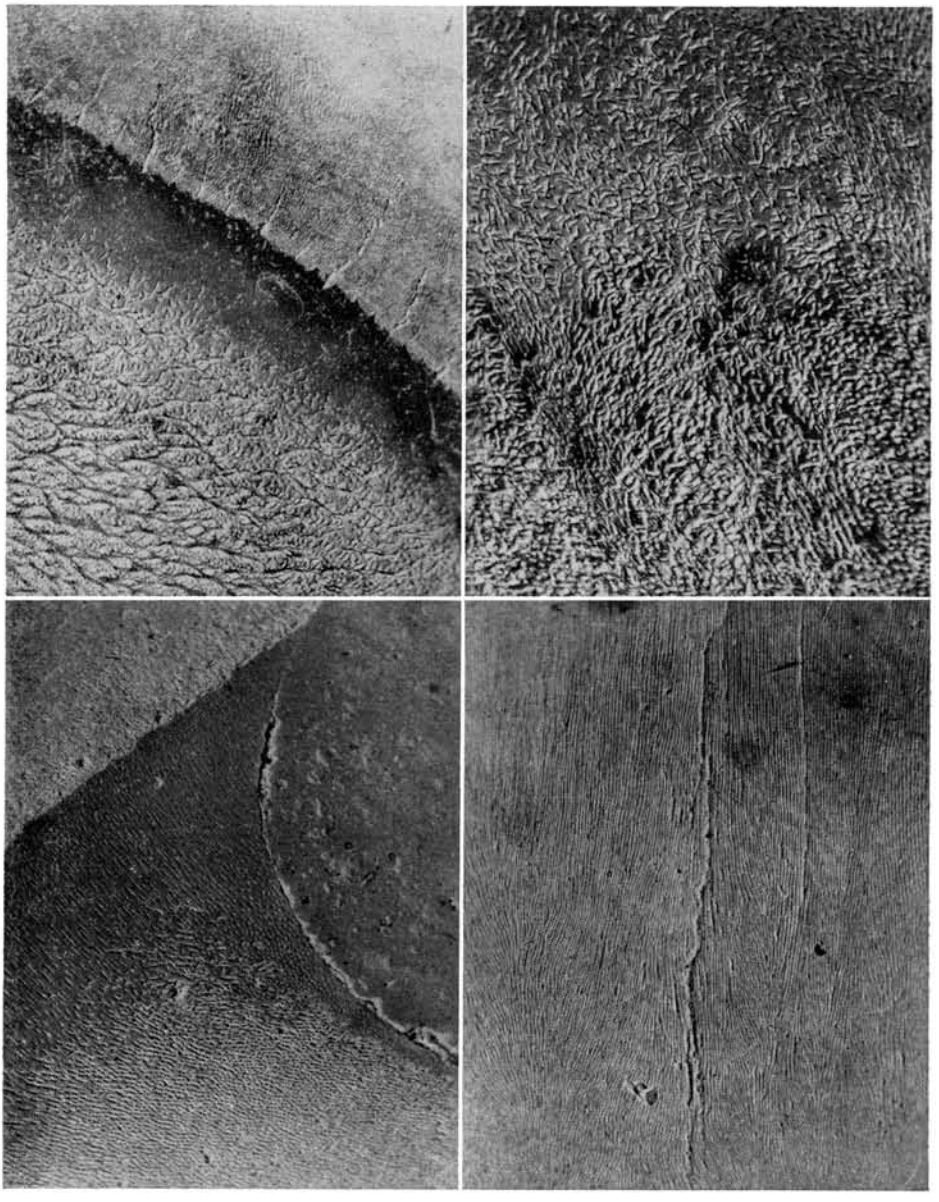

Fig. 3. Fig. 4.

Fig. 1.—Transverse section of mandibular second molar, mesial lingual cusp area. (Orig. mag. ×254.)

Fig. 2.—Transverse section of mandibular second molar, buccal area just above cemento-enamel junction. (Orig. mag. ×378.)

Fig. 3.—Transverse section of mandibular first molar, cervical area of proximal box. (Orig. mag. ×245.)

Fig. 4.—Buccal-lingual section of mandibular first molar, enamel over distal lingual cusp. (Orig. mag. ×245.)

Fig. 5. Fig. 6.

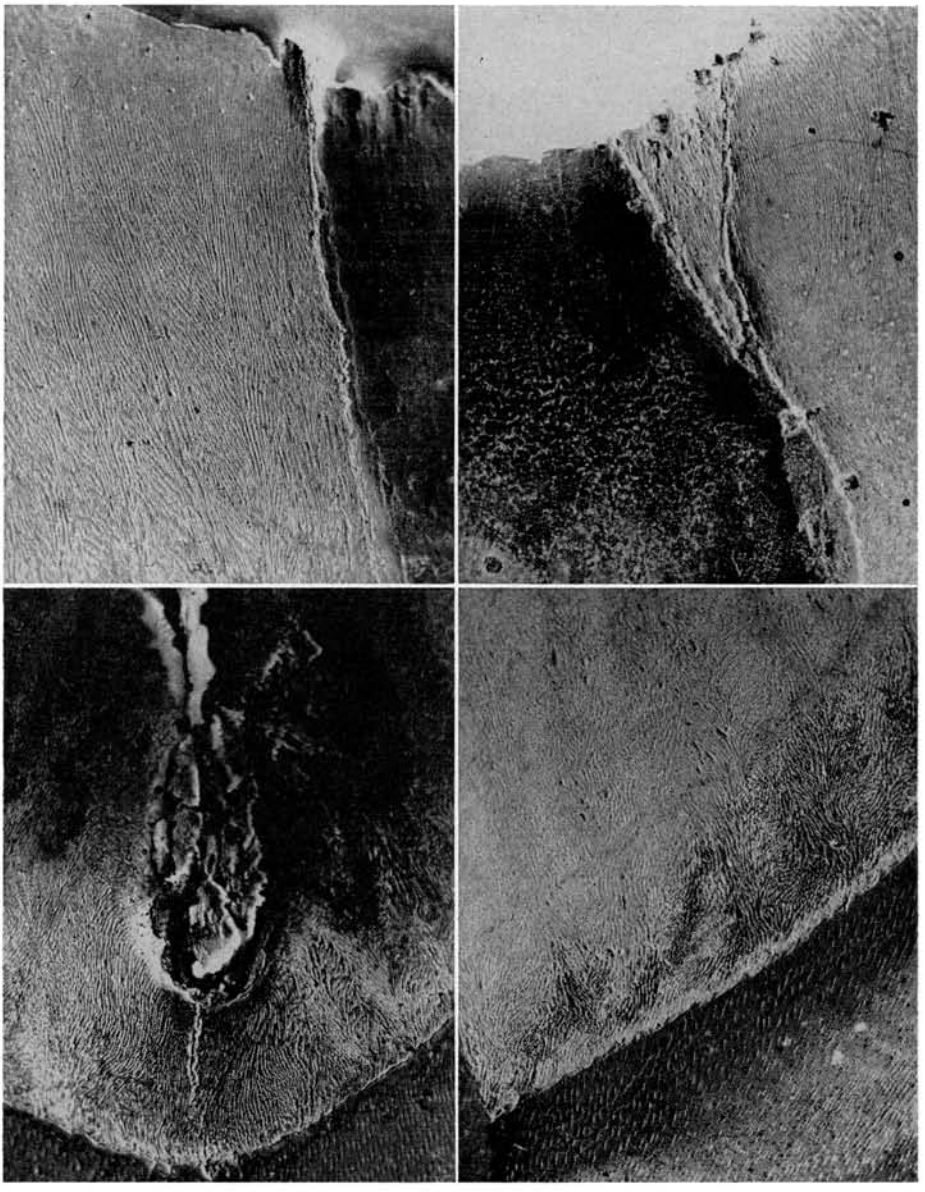

Fig. 7. Fig. 8.

Fig. 5.—Buccal-lingual section of maxillary second premolar with MO restoration through distal aspect of cusps. (Orig. mag. ×245.)

Fig. 6.—Buccal-distal section of maxillary third molar with occlusal restoration, through mesial cusps. (Orig. mag. ×245.)

Fig. 7.—Mesial-distal section of maxillary first molar, through central fossa. (Orig. mag. ×245.)

Fig. 8.—Buccal-lingual section of mandibular second premolar with DO restoration, through mesial fossa. (Orig. mag. ×245.)

Fig. 9. Fig. 10.

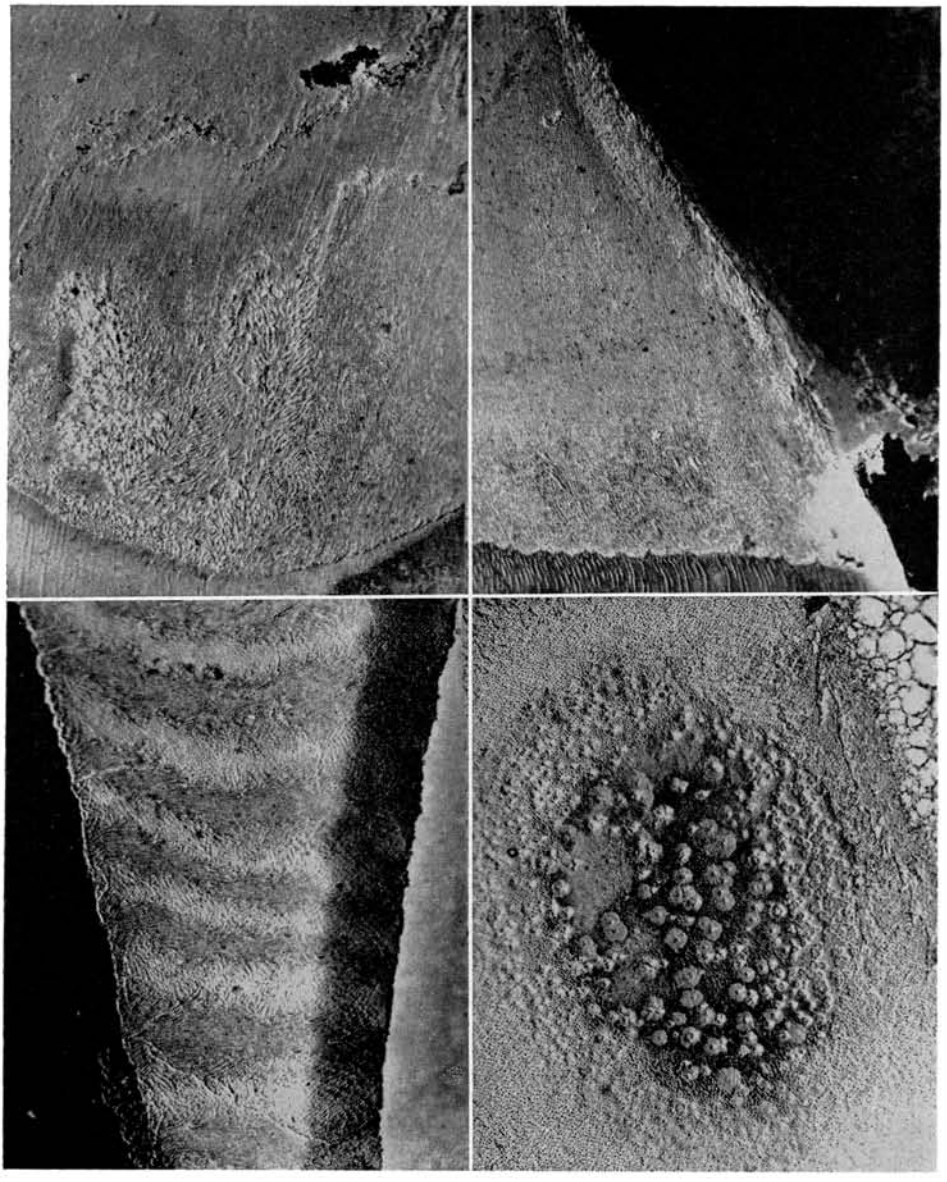

Fig. 11. Fig. 12.

Fig. 9.—Buccal-lingual section of maxillary second molar, through lingual groove. (Orig. mag. ×245.)

Fig. 10.—Mesial-distal section of mandibular second molar with occlusal restoration, through buccal cusps. (Orig. mag. ×245.)

Fig. 11.—Mesial-distal section of maxillary first molar with mesio-occlusal and an occlusal restoration, through buccal cusps. (Orig. mag. ×245.)

Fig. 12.—Transverse section of maxillary second premolar with mesio-occlusal restoration, through cervical floor. (Orig. mag. ×245.)

Hunter-Schreger bands, as observed by this histologic technic, have received considerable comment elsewhere. Fig. 11 shows the Hunter-Schreger bands in parazone and diazone formation running from the dentinoenamel junction to the surface. The dark band at the surface is probably due to a heavy metallic shadowing of that under edge of the replica, since that edge faced the electrode.

In Fig. 12 the adaptation of a cement base is demonstrated in the deep portion of the cavity. This base is separated by a thin band of primary dentin from the secondary dentin which presents an interesting pattern.

SUMMARY

A detailed technic of specimen preparation using the collodion replica method followed by metallic shadowing has been presented. This technic enables one to make rapid and accurate general examinations of the hard tooth tissues under the optical microscope. There are definite advantages gained by studying undecalcified hard tissues, yet the replica eliminates the disadvantages of observation in reflected light or the observation of thin sections with transmitted light.

The use of this technic for the histologic study of tissue surfaces is shown by the photomicrographs taken from the specimens made of teeth with amalgam restorations. The observations demonstrate a need for continued study of the traumatic effects on hard tooth tissue as a result of instrumentation.

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