Dental plaque morphology as revealed by direct observation and by replicating techniques

TRYGGVE LIE & FRANCESCO GUSBERTI

Department of Periodontology and Research Laboratories, School of Dentistry, University of Bergen, Bergen, Norway

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The ability of four elastomer impression materials to reproduce details of bacterial plaque structures was studied by comparing areas of the replica models with corresponding areas of the replicated plaque. Plaque was grown on hydroxyapatite splint segments for 48 hours and replica models were made from three different resins. One of the resins, a methacrylate material, was not suited for this purpose due to its content of spherical particles. The polysulfide and polyether impression materials reacted with the heat curing epoxy resin, and less satisfactory results were obtained with cold curing epoxy resin. Best results were obtained by a combined use of low viscosity silicone impression materials and cold or heat curing epoxy resins as model materials. However, a considerable loss of detail occurred in the replica models compared with observations of plaque directly on splint surfaces. Outlines of individual bacteria could sometimes be seen in the models, but generally several cells seemed to fuse, and appeared like small globular structures where cells and pellicle were difficult to distinguish. The bacterial colonization started near the gingival border of the surface, initiated by attachment of individual bacteria to the pellicle surface.

Key-words: Bacteriology; dental impression materials; surface morphology

Tryggve Lie, Department of Periodontology, School of Dentistry, University of Bergen, Årstadveien 17, N-5000 Bergen, Norway

The morphology of supragingival dental plaque has been the object of a number of studies. Such studies have partly used sectioning of the specimens and observation of its internal structure by light (5, 21, 24, 34) or by transmission electron microscopy (8 14, 18, 20, 33).

The clinical appearance and development of dental plaque has also been studied on photographs taken after application of disclosing agents (2, 4, 7, 12). With the great depth of focus attainable by scanning electron microscopy (SEM), additional information has evolved regarding the three-dimensional structure of plaque (3, 10, 17, 23, 25, 35). By use of this technique, plaque has been studied on the surfaces or teeth (3, 11, 23, 25), enamel cylinders (35) or hydroxyapatite splint segments (17). Replication of tooth surfaces *in vivo* has also been performed in SEM studies of plaque and pellicle formation (30, 31, 32). However, considerable discrepancy exists in the reported appearance of plaque structures between studies using a direct observation method and studies using an indirect replicating technique.

Attempts to assess the accuracy of repli-

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cating methods in relation to a direct observation in studies of dental plaque morphology will be reported. By using hydroxyapatite splint segments attached to buccal tooth surfaces and allowing plaque to accumulate, areas of the plaque-covered splint surface could be compared with corresponding areas in replica-based models.

MATERIAL AND METHODS

Hydroxyapatite splint segments were attached to the buccal surfaces of maxillary molar and premolar teeth in an adult individual with good oral hygiene and healthy gingival conditions as described previously (15). The segments remained in place for 48 hours during which time no oral hygiene was performed and no specific dietary restrictions were instituted. Replicas were then taken of the plaque that had grown on the splint surfaces during the 48 hours period.

Three "light body" elastomer impression materials, Permlastic®, Delicron® and Xantopren®, and a "regular" type of material, Impregum®, were applied according to the manufacturers' descriptions. Permlastic® is a polysulfide based material, Impregum® is a polyether material while Delicron® and Xantopren® both are silicone impression materials.

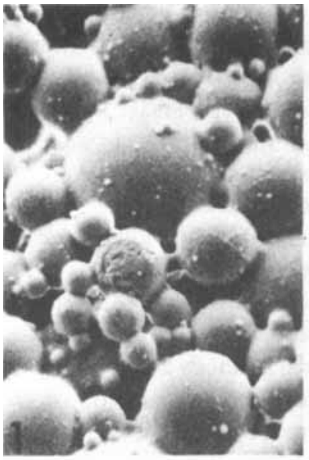
Before taking each replica, the surface was gently sprayed with a stream of water, dried with an air jet and the whole area was isolated with cotton rolls. After polymerization, the replicas were removed, rinsed in running tap water and distilled water, dried in air and placed in small aluminium trays that were poured with the model material. Three different model materials were tested: Specifix® which is a cold curing methacrylate resin, Epofix®, a cold curing epoxy resin, and Durcupan®, an epoxy resin dependent on heat for its polymerization within a reasonable length of time. Entrapped air was evacuated from the epoxy resins by vibration in an ultrasonic cleaning device, and after polymerization the positive models were ultrasonically cleaned in order to clear off adhering loose particles.

After impressions of splint surfaces had been taken, the splint segments were removed and placed in cold 10 % buffered formalin for 48 hours. At the same time, splint segments that had not been replicated were removed and included in the study for control purposes. After fixation, the splints were dehydrated in ethanol and dried in air. Splint segments and resin models were then mounted on metal specimen holders and coated with gold under continuous tilting and rotation. Scanning electron microscopic (SEM) observation of the specimens was performed in a JEOL JEM-S1 microscope operated at 10 KV and with a tilting angle of 20 or 30 degrees.

RESULTS

A total number of twelve positive models resulting from the combined use of the various replicating and model materials were compared with the corresponding plaque structures on the splint surface. It soon turned out, however, that one of the model materials, Specifix, was unsuitable for the purpose of this study due to its content of spheric filler particles (Fig. 1). Impressions were easily removed from such models, but the filler particles completely masked any detail of the surface (Fig. 2). Models made from this material thus gave a false picture of the plaque irrespective of what type of impression material was used (Figs. 2, 3).

It also turned out that two of the impression materials, Permlastic and Impregum, reacted with Durcupan during its polymerization. They adhered so strongly to the Durcupan models that they could only be separated from it with great efforts. Traces of impression material were sometimes impossible to remove completely.



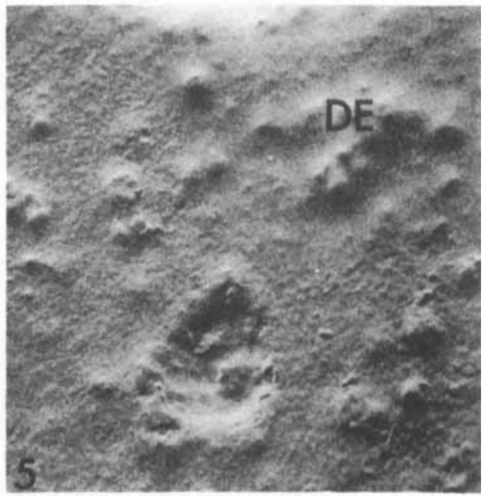


Fig. 1. Spheric filler particles in the methacrylate resin. x 480.
Fig. 2. Details from the polysulfide impression is completely lost in this methacrylate model due to protruding filler particles. x 150.
Fig. 3. Voluminous plaque on the replicated splint surface corresponding to area illustrated in Fig. 2. x 150.

Fig. 4. Plaque growth is mainly located within and around defects, DE, of this splint surface area which has been replicated by the polysulfide impression material. x 75.
Fig. 5. Part of epoxy resin model corresponding to area illustrated in Fig. 4. Note loss of details and changes of surface morphology especially relating to the defects, DE. x 75.

When Epofix was used as the model material, however, both Permlastic and Impregum impressions were removed from the models with only moderate efforts. But the reproduction of details in these models was so unsatisfactory (Figs. 4, 5) that for the remaining part of the study it was decided to use the two low-viscous silicone materials Delicron and Xantopren in combination with the epoxy resin model materials.

Both Delicron and Xantopren were easily removed from the epoxy resin models and the two impression materials as well as the two epoxy resin model materials were approximately equal with respect to their ability to reproduce details of plaque structures on the splint surfaces (Figs. 6, 7, 8, 9). Identification of individual bacterial cells in the models was, however, difficult regardless of the *magnification used*

(Figs. 11, 13, 15). In the corresponding areas of the splint surface, individual bacteria were readily recognized both at low and at high magnifications (Figs. 10, 12, 14). Plaque areas in the models were usually recognized by observing the contours of the plaque, both the border-lines of early monolayers of cells (Fig. 11) and the gross contour of more voluminous plaque structures (Fig. 15). Individual bacterial cells on the pellicle surface as reproduced in the models gave the impression of being covered by a masking coat and therefore appeared as indistinct globular structures which were difficult to distinguish from pellicle material (Figs. 11, 13).

Observation of control splint segments that had not been subjected to the impression procedures failed to demonstrate a complete masking of the plaque bacteria such as observed in the models. In some areas a layer of organic material could be seen to cover partly the organisms but generally their shape and dimensions were readily recognized (Fig. 16) and bacteria and organic material could easily be distinguished (Fig. 17).

Two types of hemispheric structures, occurring in different areas, were seen on direct observation of one of the splint specimens that had previously been replicated by Delicron (Figs. 18, 19). One type of these structures varied greatly in dimension and occurred in several areas superimposed on the pellicle surface. They bore little resemblance with bacteria or with bacterial aggregates (Fig. 18). The other type of hemispheric structures were of more uniform dimension and occurred interspersed between the bacteria on the plaque surface (Fig. 19).

DISCUSSION

The most important advantage of the indirect replicating methods as opposed to direct observation of specimens in studies of dental plaque morphology, is that the microbial structures can be observed on natural tooth surfaces without extraction of teeth. Another possible valuable advantage is that the sequential changes taking place as the plaque grows may be displayed in longitudinal observations of tooth surfaces. If such replicating methods are to represent an alternative to direct observation of the plaque, however, the methods will have to fulfill certain requirements. The impression material should have sufficiently low viscosity so that it could easily flow into and replicate the small details of the heterogenous plaque structures even at the presence of some moisture. The model material in turn, should duplicate the impression accurately without adhering to or reacting with it. The model material should also withstand the bombardment of electrons in the scanning electron microscope without alternations in volume or shape. Finally, the replicating procedures should neither remove components from nor add components to the surface studied.

None of the replicating methods applied in the present study reproduced details of the plaque with sufficient degree of accuracy when compared to the direct observation of the plaque specimens. This is in accordance with previous SEM studies of dental surfaces or surface deposits where replication of specimens (1, 28, 31, 32) have consistently given less resolution of details than direct microscopic observation, especially with regard to the resolution of plaque structures (15, 17, 23, 25, 26, 27, 35). There may be several reasons for this observation. One important factor is probably the viscosity of the impression material (1). A high viscosity is likely to give an incomplete duplication of finer details and a tendency towards confluence of such details in the

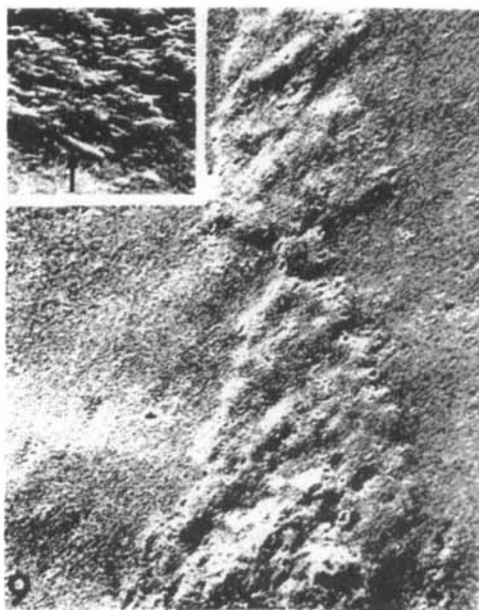


Fig. 6. Early colonization within and around a defect of the splint surface. x 720.

Fig. 7. Same area as seen in Fig. 6 after replication with silicone impression material and casting with epoxy resin. The details of the bacterial colonization are lost. x 720.

Fig. 8. Localized bacterial colonization on pellicle-covered splint surface. Inset represents another area of same specimen corresponding to inset on Fig. 9. x 2000

Fig. 9. Same area as in Fig. 8 reproduced in epoxy resin model after replication with silicone impression material. Inset demonstrates how the outline of individual bacteria (arrow) can be reproduced. x 2000

positive model. In the present study where relatively low viscosity impression materials were used, the contours of neighboring bacteria nevertheless tended to fuse, resulting in a diffuse globular pattern.

It has been claimed that Xantopren might be used with half of the manufacturer's recommended volume of soft hardener to lower its viscosity, and that this would give a limit of resolution of approximately 0.1 microns in the SEM (19). Even if this might be possible in duplication of dry, hard surfaces, it has never been demonstrated in studies of dental plaque morphology.

Loss of details by use of silicone impression materials might be due to incomplete homogenization of the silicone paste with the liquid catalyst (1). If the object of observation is not plaque structures but dental fillings or cavity margins, however, the resolution yielded by the silicone impression materials seems to be acceptable (1, 9).

Presence of moisture in the plaque is another factor which may interfere with the possibility of obtaining accurate impressions. In the present study, the surfaces to be replicated were dried by the use of an air jet, and some residual moisture is likely to have remained within the plaque. It has been demonstrated that the presence of water will prevent optimal contact between the impression material and the duplicated surface by the use of both silicone and polysulfide materials (13). Hydrocolloid impression materials on the other hand, are probably better suited for duplicating moist surfaces because of their water content (13). In studies of dental plaque morphology (28), however, such materials as yet have not proven to give results superior to those obtained with other impression materials.

The surface of dental plaque is sometimes covered by a masking layer of organic material (3, 6) although such a layer is not always present or may be present only in local areas (17, 18, 22, 35). The presence of a surface layer in the plaque specimens before the impressions were taken might explain the lack of details in the models. Comparisons between models and plaque specimens that had not been subjected to impressions, did not confirm the presence of a continuous masking layer since in general the individual organisms could readily be recognized on direct observation. One should realize, however, that the plaque observed directly by SEM had been subjected to fixing and dehydration and that some material might have been removed during these procedures.

Although the morphology of the plaque on the replicated splint surfaces did not seem to change significantly as a result of the replication, it is possible that some of the hemispheric structures observed in one of the splint segments after impression by Delicron, could represent some component of the impression material that had been added to the surface. Alternatively by-products of the impression material could have influenced the underlying organic material giving rise to morphologic artifacts. It is known for instance, that during the setting reaction of silicone polymers by-products such as methyl or ethyl alcohol are released (29). Especially those structures which showed the greatest variability in dimension, could also conceivably consist of

Fig. 10. Area of splint surface which is covered by pellicle or colonized by bacteria. x 2000.

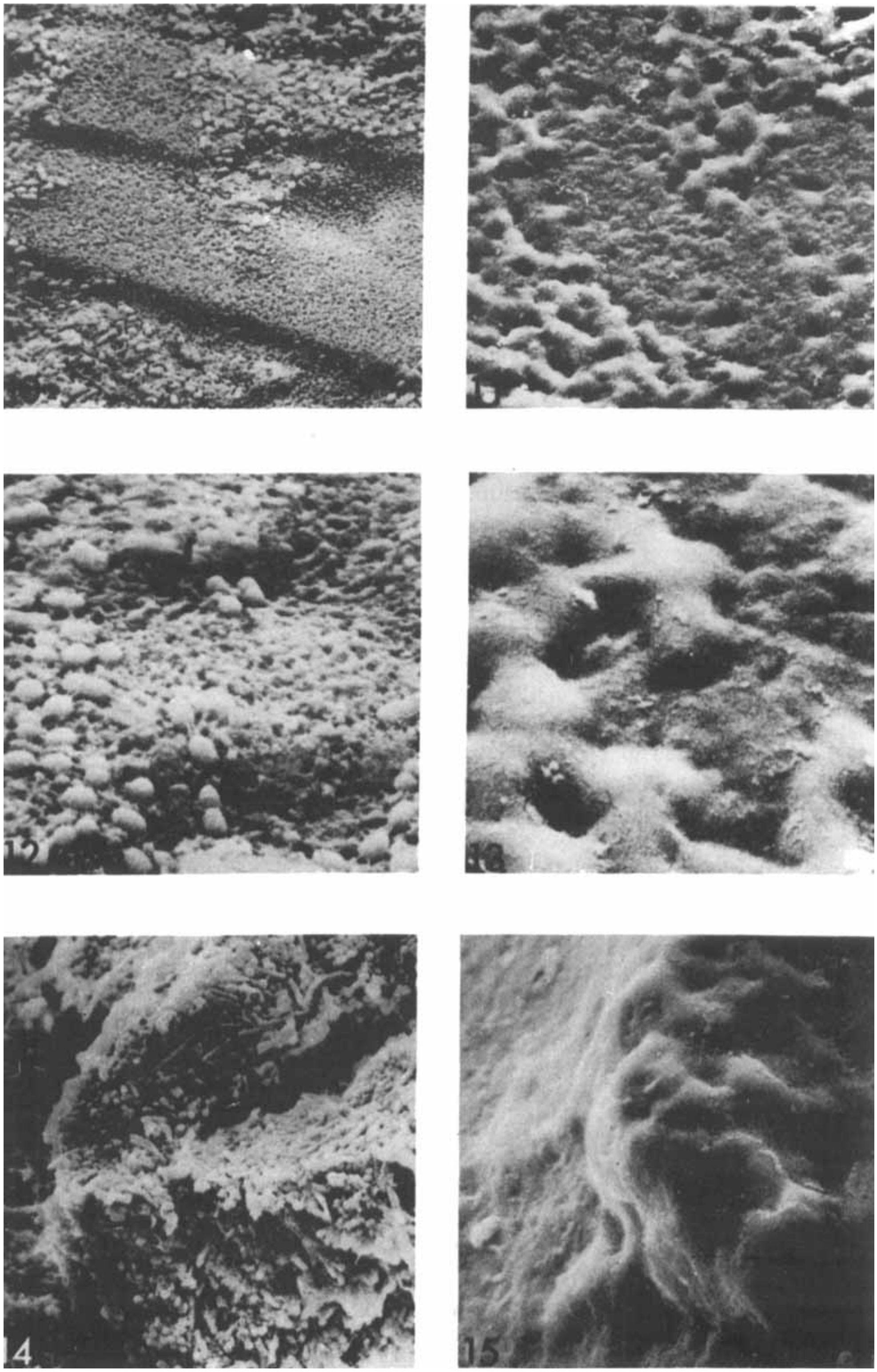
Fig. 11. Epoxy resin model area corresponding to area in Fig. 10. Replication with silicone impression material. The gross outline of the bacterial growth can readily be recognized but the details are lost. x 2000.

Fig. 12. Higher magnification of area in Fig. 10 demonstrating individual bacteria on the surface of a small globular pellicle. x 7000.

Fig. 13. Area corresponding to that in Fig. 12. Note confluence of details resulting in a globular appearance of the bacterial colonization. Silicone impression and epoxy resin model. x 7000.

Fig. 14. Voluminous plaque growth on splint surface demonstrating both cocci and rods. x 2000.

Fig. 15. In this epoxy resin model corresponding to Fig. 14, the gross contour of the plaque is reproduced but the details are lost. Silicone impression. x 2000.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mucous material from saliva, although similar structures have not been demonstrated previously (16, 17). The more uniform-looking globules that appeared interspersed between microorganisms of the same specimen, were probably leucocytes, and identical to some of the globules demonstrated by Saxton (31).

Silicone impression material has also been employed to prepare models after prior impression of dental plaque by a hydrocolloid (28). This does not seem to represent a good solution to the problem of plaque duplication as the silicone elastomer is likely to alter its volume in the SEM due to its high coefficient of thermal expansion (9). The metal conducting coat is then liable to crack and flake off as a result of small temperature changes (9). The best results seem to have been achieved by use of various resin embedding materials for the production of models (9, 31, 32).

The present study has demonstrated that silicone impression materials and epoxy resin model materials gave the most satisfactory duplication of plaque morphology, although the resolution of details was far more inadequate than by direct observation of specimens. Further studies are needed to evaluate the roles played by moisture, impression material and model material for the accuracy of the replicating techniques.

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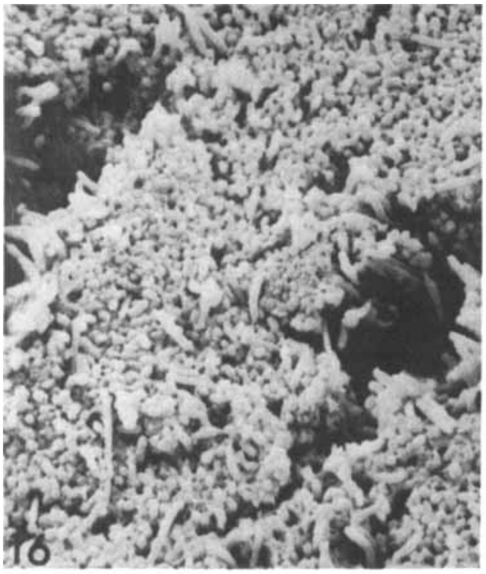

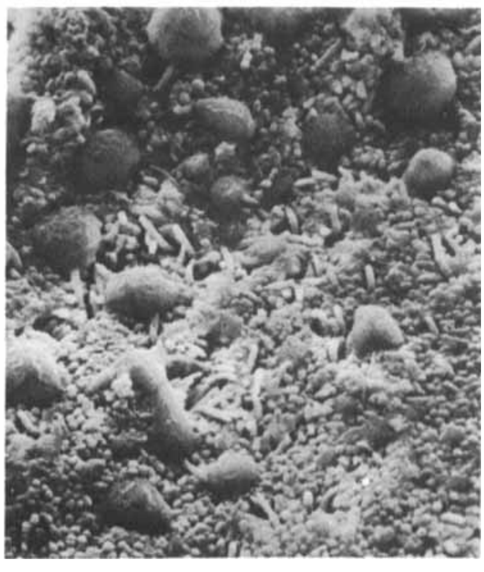

Fig. 16. Plaque morphology on control splint segment that has not been replicated. No masking layer is covering the individual organisms. x 2 000.
Fig. 17. The organic material and the individual organisms are easily distinguished in this control splint specimen that has not been replicated. x 2000.
Fig. 18. In one of the splint specimens that had been replicated with Delicron®, a number of hemispheric structures, H, occurred on the pellicle surface. They had varying dimensions and were easily distinguished from the bacteria. x 2000.
Fig. 19. The hemispheric structures interspersed between the bacteria of the plaque surface are relatively uniform in size and may represent leucocytes. Same specimen as in Fig. 18. x 2000.

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