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## Investigation of Plaque Formation by Scanning Electron Microscopy

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**Abstract:** The aim of this study was to investigate plaque formation on natural tooth surfaces by a replica technique, which offers investigation of oral hard tissue surfaces by scanning electron microscopy. This method is recommended in cases where direct investigation is impossible such as when non-

extracted teeth are investigated or when extracted teeth are expected to be influenced by the dehydration and drying process. The representative photographs demonstrate the accumulation of plaque in two days.

**Key Words:** Replica technique, scanning electron microscope, dental plaque.

### Introduction

Dental plaque is considered to be a complex, metabolically interconnected, highly organized bacterial system consisting of dense masses of microorganisms embedded in an intermicrobial matrix (1-5). A thin layer of fenestrated pellicle, which is an organic bacteria free film, deposits on the tooth surfaces within two hours after the teeth are brushed. Then the bacteria starts to colonize. The dental plaque, in sufficient concentration, can disturb the host-parasite relationship and cause dental caries and periodontal disease. Several studies have been conducted to show the changing pattern of bacteria colonization in dental plaque (1-5).

Scanning electron microscope investigations of defects and different hard tissue structures of the oral cavity have always been a problem. These structures should be removed from the oral environment to be investigated by a scanning electron microscope (SEM). However, studies have proved that it is possible to examine the surface of these tissues by creating models that duplicate oral tissues (6-14).

The direct method of investigating natural hard tooth tissues by scanning electron microscopy requires an effective fixation, dehydration and drying procedures to avoid artefacts (8, 15). It is important to apply the dehydration process meticulously so that critical point drying can be avoided with regard to hard tooth tissues.

The indirect method can be used in cases where direct investigation is impossible. For instance, when non-extracted teeth are investigated for the naked root surfaces, adaptation of the restorations, or when extracted teeth are expected to be influenced by the dehydration and drying process (6-14). The aim of the present article was to investigate the dental plaque accumulation on natural teeth surfaces by a replica technique using a scanning electron microscope.

### Materials and Method

A female with healthy maxillary incisors volunteered to accumulate plaque for the SEM investigation. All her teeth were first professionally cleaned using Nupro cleaning pastes, applied in a rubber cup rotated at slow speed. The surfaces were rinsed with water, followed by spraying with air and water. The impression was then taken immediately. The subject was instructed to avoid oral hygiene procedures for 2 days in which 2 more impressions were taken (24h- and 48h-plaque).

The teeth were dried with air before taking the impressions. Provil (Heraeus Kulzer, Dormagen, Germany) was used as an impression material. A thin layer of the impression material was adapted to the teeth surfaces by gentle air blow and the material was thickened and gently flexed off the teeth after it set. The impressions were kept in a sealed dust-free container for

at least 24 hours. After they were rinsed with alcohol, the impressions were boxed, filled with epoxy resin (Stycast, National Starch and Chemical Company, Westerlo-Belgium) and kept in vacuum for 30 minutes. They were cured for 24 hours at room temperature. After separating from the impressions, the replicas were coated with a thin layer of gold by a Sputter-Coater (Agar Scientific Ltd. 'Model 103 Essex, England) and blindly examined with a scanning electron microscope (Amray Inc. '1610' Bedford-Massachusetts USA) and representative photographs were taken.

## Results

The subject had healthy incisors and accepted to accumulate plaque for two days. Replica models taken from the subject were coated with gold and investigated in SEM by the same researcher. The aproximal region after professional tooth cleaning is seen in Figure 1a and 1b at a magnification of x500 and x1000. The buccal surface of the incisor is seen in Figure 2a and 2b at a magnification of x500 and x1010 respectively. The scanning electron photomicrograph from the clean tooth

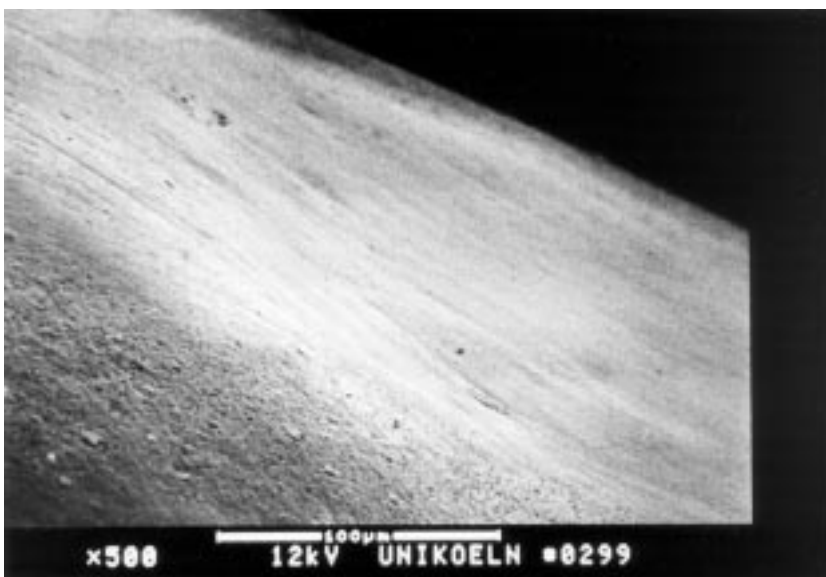


Fig. 1a. The aproximal region after professional tooth cleaning on replica model (SEM x500).



Fig. 1b. The aproximal region after professional tooth cleaning on replica model (SEM x1000).

surface revealed perikymata with cleaning paste remnants on the surface. Figure 2b shows a piece of the cleaning paste remnant at a magnification of x1010.

The remnants of the cleaning paste and colonization of plaque after 24 hours were clearly replicated (Figure 3a and 3b). The plaque started to accumulate and mask the perikymata on the enamel surface. After 48 hours, there was a thick layer of plaque accumulated on the buccal surfaces of the incisors on replica models (Figure 4a and 4b). These photographs represent a continuous layer of plaque by adherence and bacterial growth on the tooth surface.

### Discussion

Dental plaque as an adherent mass of bacteria covering tooth surfaces can be removed by brushing (16). In general, bacterial attachment to acquired pellicle on the tooth surface causes plaque to accumulate and thicken but the forces that bind plaque to the surfaces are not very strong. This makes the investigation of plaque by scanning electron microscopy harder. Christ (3-5) investigated the plaque on extracted tooth surfaces by scanning electron microscopy and showed different types of bacteria under very high magnifications. There were



Fig. 2a. The buccal surface after professional tooth cleaning with cleaning paste remnants on replica model (SEM x500).

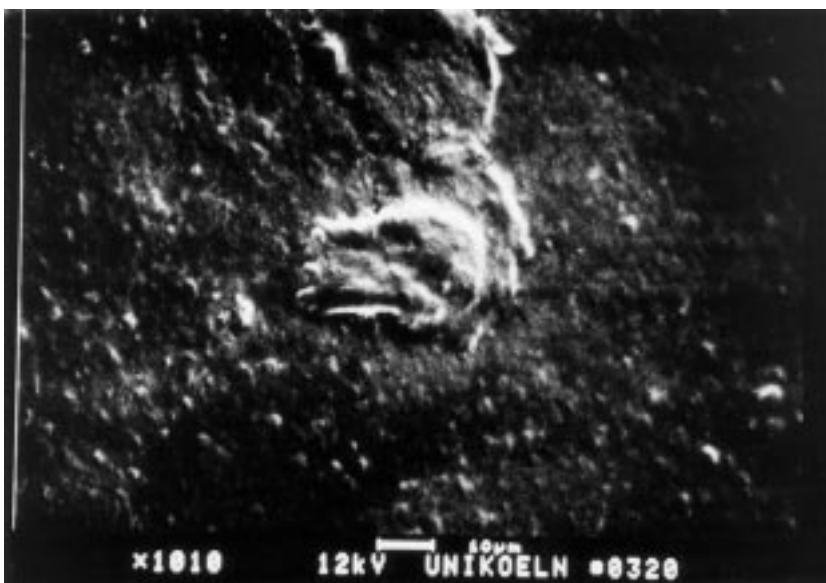


Fig. 2b. The buccal surface after professional tooth cleaning with cleaning paste remnants (SEM x1010).

erythrocytes of blood due to extraction and plaque was damaged (3-5).

A series of dehydration procedures and coating with gold is necessary to investigate surfaces by scanning electron microscopy. These procedures may affect the texture of the investigated surface so the indirect method is used by taking impressions of the tooth surfaces with an addition type polysiloxane. A positive model is manufactured from this impression using an epoxy resin (6,7,9,15). Indirect method using replica models, helps to investigate non-extracted tooth surfaces by SEM.

Ulusu et al. (8) investigated dentinal adaptation of resin composites, Gaengler et al. (13) studied the anti-calculus efficacy, whereas Brady and Woody (14) examined cervical erosion by replica models. Absi et al. (11) and Rimondini et al. (12) studied dentine hypersensitivity by the replica technique. Such studies are necessary and precede the clinical investigation since they permit replication and comparison under ideal conditions. Ulusu et al. (8) stated that observation of replicas was useful to eliminate the inclusion of false gaps on tooth surfaces due to the dehydration process of natural teeth in the



Fig. 3a. The accumulation of plaque after 24 hours at a magnification of x500 (SEM).



Fig. 3b. The accumulation of plaque after 24 hours at a magnification of x1000 (SEM).

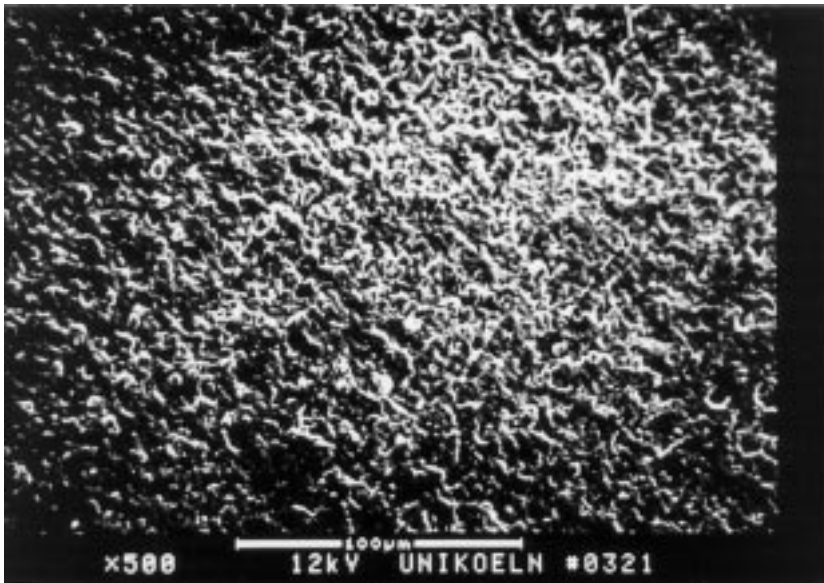


Fig. 4a. The thick layer of plaque accumulated after 48 hours, on the buccal surfaces of the incisors on replica models (SEM x500).

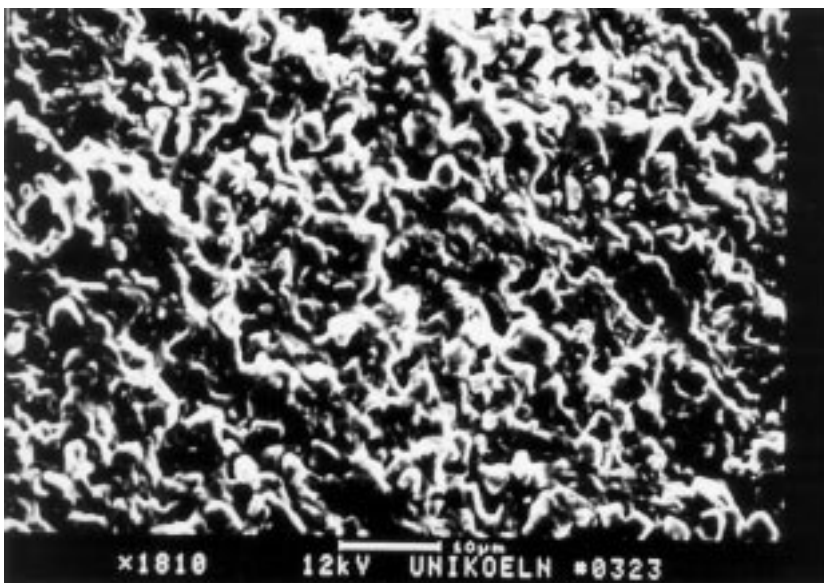


Fig. 4b. The thick layer of plaque accumulated after 48 hours, on the buccal surfaces of the incisors on replica models (SEM x1810).

analysis. They advise the combination of direct examination with the replica technique to get more accurate results (8).

Plaque thickness differs depending on the locally prevailing oral cleansing forces, oral hygiene, and other factors such as salivary components. It was possible to examine even a thin layer of remnants and plaque using this technique. However, within two days a continuous bacterial layer can be formed by further adherence and lateral in situ bacterial growth (3-5, 16). This was apparent in Figure 4a and 4b.

These findings demonstrate that dental plaque starts to accumulate soon after tooth cleaning. Plaque masks the perikymata on the enamel surfaces and a continuous layer is formed in 48 hours. The replica technique, using modern impression materials and SEM, offers a simple and effective investigation of surface micromorphology of the hard and soft tissues. Further studies that use the replica technique in combination with the direct method may be conducted to show the composition of dental plaque in detail.

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