Auger electron spectroscopy of iron in dental pellicle from stainers and non-stainers

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Nordbø H, Skjørland KKr, Eriksen HM. Auger electron spectroscopy of iron in dental pellicle from stainers and non-stainers. Acta Odontol Scand 1984;42:37–40. Oslo. ISSN 0001-6357.

Stainers and non-stainers were selected on the basis of their individual tendency to develop extrinsic tooth discolorations from a chlorhexidine mouth rinse. Pellicle was allowed to form in vivo on dental enamel slabs in both stainers and non-stainers. The slabs were then exposed to chlorhexidine 0.2% and a neutral iron salt solution extraorally either once or until a brownish discoloration appeared from the stainers. The pellicle was examined for its content and distribution of iron by Auger electron spectroscopy (AES). Pellicle from stainers showed higher concentrations of iron than pellicle from non-stainers. ☐ *Chlorhexidine; organic coatings; tooth discoloration*

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Previous investigations have demonstrated the presence of large amounts of iron in chlorhexidine-discolored pellicle (1–3). Staining of similar appearance has also been produced experimentally in vivo by rinsing with denaturing agents and iron solutions (1, 2). Previous in vitro studies of iron adsorption to saliva-coated hydroxyapatite were, however, unable to reveal any clear difference in iron uptake between stainers and non-stainers in a single-step experiment (4). The aim of the present investigation was to study the adsorption and distribution of iron in dental integuments by Auger electron spectroscopy under the following conditions:

1. 2-h pellicle formed in vivo and then exposed once to chlorhexidine and iron extraorally (single-step experiment)
2. Pellicle formed in vivo and intermittently exposed to chlorhexidine and iron extraorally until staining occurred.

Materials and methods

Slabs of enamel were cut from freshly extracted third molars, polished with pumice, and fixed with wax in shallow depressions on the buccal surfaces of acrylic appliances carried in the maxillary lateral region of the oral cavity. Slabs carried by subjects to be directly compared were always taken from the same tooth. In the single-step experiment, pellicle was allowed to form during 2 h in six volunteers, three selected stainers and three non-stainers, on the basis of their response to oral rinsing with chlorhexidine. The slabs (two from each participant) were then immersed in 0.2% chlorhexidine gluconate for 1 min with gentle stirring and subsequently rinsed thrice with distilled water. The specimens were then exposed to either 1 mM or 10 mM iron^{3+} ammonium citrate (Norsk Medisinal Depot, Oslo, Norway), pH 7.1, for 1 min and rinsed thrice with water before being dried by a gentle air blast. Fresh solutions were always used. One untreated slab from each person served as a control.

In the long-term experiment the appliances were taken out every 2nd h and immersed for 1 min in 0.2% chlorhexidine, followed by water rinsing and application of 10 mM iron solution for 1 min. The slabs were kept in the carrier's saliva at meals to avoid any dietary influence and at night to avoid drying. Twenty exposures were necessary to produce a marked brownish discoloration in the heaviest stainer. The pellicle on the enamel slabs were analyzed for deposition and distribution of iron and certain other elements such as carbon, calcium, nitrogen, phosphorus, and sulphur by means of Auger electron spectroscopy (AES) in a fully automated Auger microprobe (Varian Associated, Palo Alto, Calif., USA).

As the enamel slabs are non-conductive, they were mounted in a manner permitting the primary electron beam to be directed almost tangentially to their convex surfaces—that is, at a very small angle. This made it possible to reduce the charging of the sample to be analyzed, without using gold coating. Care was taken to avoid contamination of the specimens.

The analyses were performed by ELAB (Electronics Research Laboratory, University of Trondheim, Trondheim, Norway). AES was used in conjunction with argon ion sputtering to obtain depth profiles of the integuments. The sequential sputtering time was 1/10 and 1 min, and an argon ion beam of 3 keV energy and 100 μA cm^{-2} ion current density was used. The presence of carbon, nitrogen, and oxygen was taken as parameters of the existence of an organic film. The primary electron beam energy used was 2 keV, and the secondary electrons were analyzed in the range of 15 to 1000 eV.

The electron beam was stationary during the measurements, and the beam diameter was 30 μm. The argon beam and the mounting table were kept in the same position during the analyses, making it possible to obtain the spectrum from exactly the same area after each sputtering cycle. SEM pictures were used as control.

Results

The pellicles formed on the enamel slabs in the single-step experiment were sputtered off in less than 2 min. This indicates a very thin organic layer. Iron was found in all samples, but in considerably higher amounts from the stainers than from the non-stainers (Fig. 1). Moreover, the iron was more unevenly distributed in the pellicle from the stainers.

In the long-term experiment the sputtering time varied from 10 to 30 min, indicating a marked increase in thickness of the pellicle (Fig. 2). A brownish discoloration was visible on the enamel slabs from stainers. In this experiment also the depth profiles revealed significantly higher amounts of iron in stainers than in non-stainers. The iron was again found in intermittent peaks in stainers, whereas it was more evenly distributed in non-stainers.

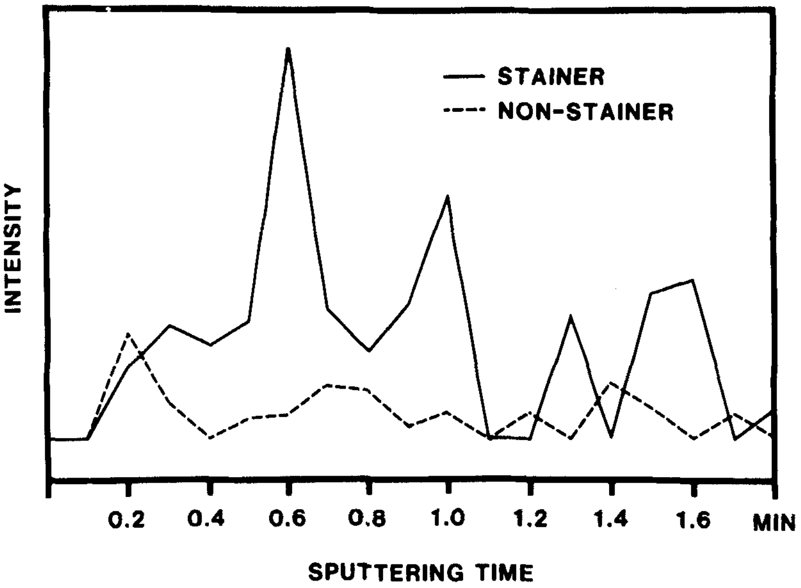

Fig. 1. Tracings of depth profiles of iron (Fe) in 2-h pellicle formed on enamel slabs from stainer and non-stainer. The pellicle was exposed to 0.2% chlorhexidine and 10 mM Fe^{3+} ammonium citrate for 1 min each before the AES analysis. The curves show the average intensity of Auger lines as a function of sputtering time. The left side of the diagram represents the outer surface of the pellicle. Higher amounts of iron are found among stainers than non-stainers.

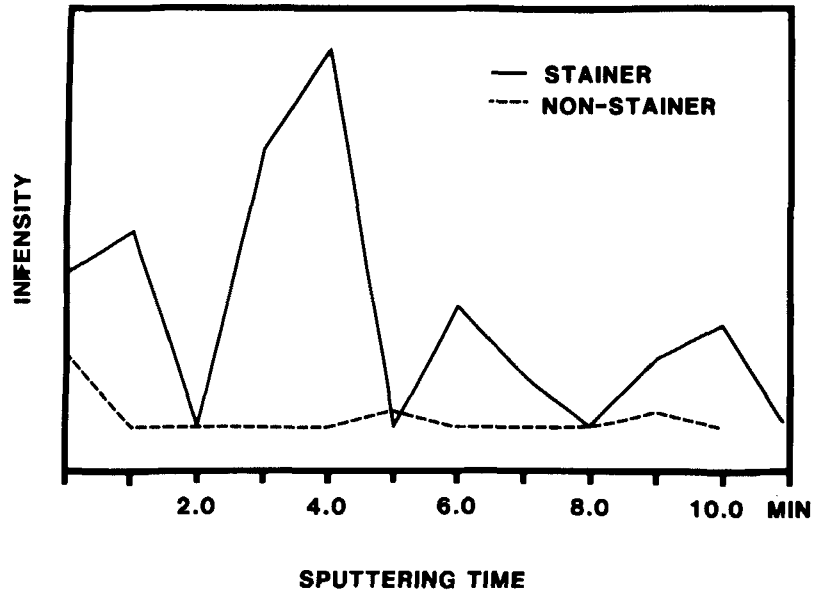

Fig. 2. Tracings of depth profiles of iron (Fe) in pellicle formed on enamel slabs from stainer and non-stainer during 5 days. The slabs were taken out four times a day and exposed to 0.2% chlorhexidine and 10 mM Fe^{3+} ammonium citrate for 1 min each. The specimens were kept in the carriers' own saliva at meals and at night. The curves show the average intensity of Auger lines as a function of sputtering time, the left side of the diagram representing the outer surface of the pellicle. A long sputtering time indicates a thick pellicle. More iron distributed in distinct 'layers' were found among the stainers.

Variations in the amount and distribution of the elements Ca, P, and S were also observed.

Discussion

The AES analyses are based on characterization of the secondary electrons emitted from the material. The escape depth of these electrons—that is, their mean free path—depends on their energy and, to some extent, on the density (molecules/cm^3) of the material to be analyzed. It is known that the manner of molecular packing of the adsorbed organic material may vary from surface to surface (5). The choice of instrumental settings for the analyses, such as primary electron beam parameters, was based on experience with known materials to minimize sample damage and charging. Depth of analysis was estimated to be approximately 0.5 to 1.5 nm. The sputtering rate of biological materials is not known. The film thickness is therefore only referred to as the sputtering time used to remove the layer or layers in question. It may be assumed to be approximately 10 nm/min for the ion beam parameters used. It is essential that the ion-induced damage is as little as possible, but it is also important to obtain a reasonable sputtering yield. The present technique has been shown not to lead to severe damage in other materials (6) and has been used in organic matters with acceptable results (7). There was no evidence of elementary carbon being produced during the sputtering with argon ions. No sudden increase in the intensity of the carbon signal or intermittent peaks were observed.

Since all subjects to be compared carried enamel slabs cut from the same tooth, any differences in the pellicles formed could hardly be attributed to variations in the enamel surface. Some variation in the amount and distribution of the elements sulfur and phosphorus was observed, indicating differences in the chemical composition of the organic films adsorbed. However, the significance of these findings is uncertain, and more detailed studies are required before firmer statements can be made.

The increased binding of iron to the pellicle material in stainers in the single-step experiment seems contradictory to the results recently obtained with saliva-coated hydroxyapatite (4). In that study samples from stainers and non-stainers showed approximately the same iron-binding capacity in a single-step adsorption experiment. This discrepancy may possibly indicate certain differences between the organic film formed on enamel and that adsorbed to hydroxyapatite in vitro.

The higher content of iron in the pellicle from stainers during the long-term experiment may indicate that more binding sites are available for the metal in these subjects. This may be due either to variations in the total amounts of potential sites—that is, quantitative variations—or to different denaturing characteristics of the pellicle material. Variations in the sulfur and phosphorus profiles between the two groups seemed to correlate with the amount of iron and may therefore support the former suggestion (1).

The distinct peaks representing iron deposition demonstrated in stainers, particularly in the long-term experiment (Fig. 2), may indicate a 'sandwich' structure of the integuments formed. This characteristic feature was not present in non-stainers.

Although the number of subjects was relatively small, the same trend was consistently found. In addition, pilot studies including other groups of persons have yielded similar information. The results obtained thus give support to the hypothesis previously suggested (1–3) that incorporation of iron may be a major factor in pellicle staining.

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Received for publication 8 September 1982