A Straightforward Scanning Electron Microscopy Technique for Examining Non-Metal Coated Dental Hard Tissues

S. M. McCormack  
*Eastman Dental Center*

F. J. Tormo  
*Eastman Dental Center*

J. D. B. Featherstone  
*Eastman Dental Center*

Follow this and additional works at: https://digitalcommons.usu.edu/microscopy

Part of the Biology Commons

Recommended Citation
Available at: https://digitalcommons.usu.edu/microscopy/vol5/iss1/25

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
A STRAIGHTFORWARD SCANNING ELECTRON MICROSCOPY TECHNIQUE FOR EXAMINING NON-METAL COATED DENTAL HARD TISSUES

S.M. McCormack*, F.J. Torma and J.D.B. Featherstone

Department of Oral Biology, Eastman Dental Center, 625 Elmwood Avenue, Rochester, NY 14620, USA

(Received for publication June 7, 1990, and in revised form October 20, 1990)

Abstract

Modifications to the standard operating settings for accelerating voltage, condenser lens current, scan rate, working distance and tilt on the conventional scanning electron microscope (SEM) enabled non-metal coated dental hard tissues and synthetic apatite pellets to be viewed free of charging effects. Well-resolved images at magnifications as high as 35,000x were achieved using accelerating voltages less than 5 kV. The methodology detailed here allowed for serial SEM examination of the same sample at various points during an experimental procedure, and may be applied to other sample types. The procedure is non-destructive to the sample and requires no physical modification to the microscope.

Key words: Scanning electron microscopy, dental enamel, apatite, low voltage, specimen coating, charging.

Introduction

Conventional scanning electron microscopy (SEM) has been used extensively to examine dental hard tissues (see, for example, Holmen et al., 1985; Martin et al., 1988; Hattab et al., 1988; Boyde et al., 1988). Typically, a thin layer of heavy metal coating is sputtered onto the sample surface to eliminate charging and increase secondary electron yield. In many instances no further experimentation is planned for the sample and coating with metals is not a problem. There are cases, however, where it is advantageous, and even necessary, to view the sample uncoated. One such example, is a serial SEM examination of dental enamel at various points during an experimental procedure. Natural dental enamel can vary significantly between samples; therefore, examination of the same enamel surface would be valuable, rather than having to use multiple samples. Complete elimination of the conductive coating, combined with the use of low (<5kV) accelerating voltage has been reported previously, but magnification was generally limited to less than 500x and the procedure was not described in detail (Howden and Ling, 1974; Lester et al., 1988). The examination of dental hard tissues at magnifications much higher than 500x is necessary in order to study the fine structure and, in particular, changes in this structure during serial experiments. Although field emission and analytical SEMs are capable of excellent resolution even at low accelerating voltages, these types of sophisticated equipment are not readily available to many users. Therefore, the intent of this study was to refine and describe in detail a technique for eliminating charging effects and achieving good resolution at intermediate magnifications when viewing uncoated insulating samples in a basic, conventional SEM.

Materials and Methods

Sample Preparation.

The samples examined included non-metal coated human tooth crowns, lased enamel, pressed carbonated-apatite pellets, and sintered carbonated-apatite pellets (see Featherstone et al., 1983; Nelson et al., 1989; Ellies et al., 1988).
A variety of common SEM adhesives were used to mount the samples, including conductive carbon paint, silver paint, aluminum tape, and non-conductive double-sided tape, as well as aluminum foil. A comparison was made of the effect of these adhesives on enhancement of conductivity. On some samples, silver double-sided tape, as well as aluminum foil, were wrapped around some samples with only the window of interest remaining exposed. All samples were room air dried before being observed in the SEM.

SEM Methodology.
A JEOL JSM-820 SEM, with an accelerating voltage range of 0-30 kV incremented in 0.1 kV steps to 3.0 kV, and 1.0 kV steps from 3.0 to 30 kV was used. Examinations of the specimens were made using either a LaB$_6$ crystal filament, or a conventional tungsten wire filament. Viewing and photo scan rates were variable.

Adjustments were made, as described below, to the standard operating settings for accelerating voltage, condenser lens current, scan rate, working distance, and tilt in order to balance the charge and optimize the resolution achieved for each sample.

Accelerating voltage. At the high accelerating voltages (15-30 kV) generally used to examine coated samples, the image of a non-metal coated sample is quickly distorted by charging effects, such as bright glowing spots, streaking, beam displacement and image movement. Therefore, we began with a very low accelerating voltage, 1.5 kV, and increased the electron beam to scan for a few seconds at slow speed and high magnification. The scan was switched to TV rate and the magnification quickly reduced to observe the type of charging effect as well as its rate of dissipation. If the scanned area was darker than the rest of the sample image, "positive charging" was occurring and the accelerating voltage was increased, in small increments of 0.1 to 0.5 kV. At such low voltages, an increase of a full 1.0 kV may cause rapid charge buildup. Conversely, when the scanned area appeared brighter than the rest of the image, "negative charging" was occurring due to the lack of a conductive pathway for the excess electrons. Accelerating voltage was then reduced in small increments. This procedure was repeated until no charging effect was observed.

With both types of charging, the rate of dissipation of the charge was a key in determining how close we were to balancing the charge. The faster the charge dissipated, the closer we were. It was important to start at a lower accelerating voltage and gradually increase the kV rather than starting at too high a kV and putting a charge on the sample that is not readily dissipated. Once the charge was balanced, the sample could be viewed indefinitely, free of charging effects.

Condenser lens current. Another important step in achieving maximum resolution with uncoated samples was to increase the condenser lens current. Increasing the condenser lens current results in a narrower beam striking the sample, thus improving resolution. Another result is reduced probe current, which has 2 effects: 1. reduced rate of charge buildup on the sample surface, and 2. a lower signal to noise ratio. To compensate for the "noisy" image resulting from the lower S/N ratio, the photographs were taken at a slower scan rate (100-300 sec/frame), thus increasing the S/N ratio and reducing the grainy appearance in the micrograph. Further noise reduction can be achieved in prints from negatives by using the "soft focus" technique of Peters (1985).

Scan rate. Although a slow scan rate is necessary for photography it can also increase the rate of charge build up on the sample surface. Therefore, choosing the region of interest, coarse focus and stigmation were adjusted while viewing in the faster TV scan rate. The scan rate was slowed for fine focusing and photography.

Working distance. At long working distances the electron beam, especially one of low kV, is subject to broadening and fluctuations resulting from stray magnetic fields, which diminishes resolution. Therefore, the use of a short working distance (8-15 mm) was found to be necessary under these conditions.

Tilt. Although tilting the sample toward the detector does not improve resolution, it is sometimes found to be helpful in reducing charge buildup and enhancing secondary electron yield. However, the combination of low kV and short working distance diminished the depth of focus capabilities of the SEM. As a result, the dynamic focus control could not compensate for the large depth of field created by final tilt angles greater than 30°, thus negating the advantages of tilt. On the mainly flat samples used in this study, a 0° tilt was found to be optimum.

Anode position. The JEOL JSM-820 SEM is equipped with a dual position anode. At low (<15 kV) accelerating voltages with a tungsten filament, the upper anode position is used to increase emission current and reduce stray magnetic field fluctuations. With the LaB$_6$ filament, the anode should be kept in the lower position at all times.

Objective aperture diameter. The use of a small diameter (50 µm) objective aperture enhanced the resolution of the image at low kV and reduced charge buildup on the sample.

Results and Discussion
For most samples, the type of adhesive used did not significantly affect the results. No particular adhesive consistently produced better results than another. Therefore, the use of the adhesive most compatible with the sample type of interest is recommended.

Accelerating voltage and probe current were the most significant parameters in controlling charge accumulation on the samples. In general, 2-4 kV was found to be the optimum accelerating voltage for viewing non-metal coated dental hard tissues and carbonated apatite pellets. Achieving charge balance was more important in obtaining good images than attempting to operate at a higher than necessary accelerating voltage.

The degree of success was somewhat affected by the sample, as is true even with metal coated samples. Of the samples used here, pressed apatite pellets were the most difficult to view. Using 2.5 kV, well resolved images could be achieved up to approximately 10,000x. Greater
SEM examination of non-metal coated samples

success was achieved with ceramic apatite pellets, with no appreciable loss of resolution occurring at magnifications up to 20,000x. Figures 1a and 1b are comparative micrographs of metal coated (150Å of gold and palladium) and non-metal coated ceramic apatite pellets viewed at 10,000x using 30kV and 3.0kV, respectively.

Figures 2a and 2b are micrographs of non-metal coated lased enamel viewed with accelerating voltages of 3.0kV and 4.0kV at magnifications of 15,000x and 30,000x. This sample was part of a serial study and was our impetus for exploring the resolution limits of non-metal coated samples at low kV. Although there is some loss of resolution at the higher magnifications, there is still considerable information to be gained from these micrographs.

We have successfully viewed several other non-metal coated samples, e.g. corrosion products on nylon test fabrics, particulates air dried onto gold/palladium coated glass cover slips, a cross-section of a tooth embedded in plastic, and cross-sections of carious lesions in dentinal tissue. Further examples which have been suggested where a metal coating might even be prohibited include rare archaeological artifacts, palaeontological specimens, and samples used as evidence in a legal proceeding.

In conclusion, we have shown that using this technique for viewing non-metal coated samples at low kV, it is possible to achieve good resolution at magnifications much higher than previously reported. This technique is basic and non-sample specific and can in principle be applied to virtually any sample type. The method is particularly useful for dental hard tissues and related minerals; and we feel that its greatest usefulness is in the area of serial studies.

References


Ellies LG, Carter JM, Natiella RJ, Featherstone JDB,


Oldershaw MD (1983). Acid reactivity of enamel samples during serial SEM examinations. Chemical measures of changes during serial experiments with dentin were the same with or without SEM observations.

R.P. Apkarian: Has there been a concern or is there evidence of lateral diffusion of silver paint into the exposed surface area under observation either during painting or imaging procedures?

Authors: We have not observed any distortion of dentin or enamel samples prepared at controlled pH and temperature. In Hayat MA, Principles and Techniques of Scanning Electron Microscopy. Van Nostrand Reinhold, New York. 149-158.


Discussion with Reviewers

J.D. Fairing: The well known technique of wrapping a specimen tightly in a thin metal foil, cementing the foil to a stub with conductive glue, then piercing a small hole on the foil at the point of observation might eliminate the need for silver paint. Please comment.

Authors: Generally, the types of samples that we work with require that a larger surface area be exposed in order to get an overall view of the effects of treatments. Since these samples have usually already been painted with nail varnish, the additional use of silver paint is not a problem.

A.A. Mills: How does your technique for viewing uncoated samples compare with the "removable silver film" method of Mills (1988)? Would not the latter permit use of greater accelerating voltage, magnification and resolution whenever these were required?

Authors: The application and removal of a silver film would add an extra variable to any experimental procedures being examined. We feel that this is unnecessary if the uncoated technique provides adequate results.

J. Wefel: Enamel, dentin or cementum have significant portions of an aqueous phase. How does the vacuum affect this sample and continued use as a serial sample?

Authors: We have not observed any distortion of dentin or enamel samples during serial SEM examinations. Chemical measures of changes during serial experiments with dentin were the same with or without SEM observations.