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Studies of Three Dimensional Analysis of Early Carious Lesions of Human Enamel

1. A Quantitative Observation with a Computerized Image Analysis System

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Abstract: The dual value picture and contour map capability of a recently developed image analysis system was used to quantitatively determine the degree of calcification and to study the initial changes in the area just beneath the superficial layer of early carious lesions. This method was found to easily produce a semi-quantitative analysis and is useful in describing the initiation and spread of carious changes. Moreover, this method advances knowledge of the three dimensional nature of carious change over information previously obtained by serial sections.

Introduction

In order to better understand how to prevent caries we need to advance our knowledge of the initiation, spread and recalcification of dental caries through a detailed analysis of the carious process. Histopathological studies of initial carious changes in human enamel have been based mainly on cut-surface observations. This destructive method of analysis is unable to provide complete information on the overall morphology of the carious process.

The authors previously reported on the relationship between macroscopic findings and the microradiographic appearance of the subsurface layer of white and brown spots on teeth1,2. They described considerable difference between the two types of lesions and called attention to the difficulty of macroscopic evaluation of early carious changes.

In order to quantitatively assess the extent of calcification of the subsurface lesions and to analyze the initial stage of the carious process, serial sections were studied using quantitative assessment of the dual value picture and contour maps produced by a recently developed image-analyzing system. This provided a basic study in the stereoscopic assessment of the location and degree of calcification in these lesions.

The results of this study were then compared with findings obtained from linear analysis of densitometry studies commonly used in these investigations.

Methods

The maxillary right second molar of a 36 year old female was studied. Prior observation of the tooth under stereoscopic microscopy revealed mild brown spots on the lingual surface, without recognizable carious activity. Serial sections approximately 100 μm thick were prepared following the method previously describ-
Fig. 1 A diagram of EXCEL II system

ed by the author\textsuperscript{13}. Microradiographs of these sections were exposed along with a reference of 29 sheets of aluminum of 11 \( \mu \)m thickness each. A CMR–2 ultrasoft X-ray machine (Softex) was used with a film-focus distance of 6 cm, a setting of 15 kV and 4 mA, and an exposure time of 10 minutes as previously described by the author\textsuperscript{40}.

Kodak film No. 649–0 was used and was developed according to manufacturer’s directions.

The same specimen was then subjected to image analysis using a computer. An Image Analysis System, EXCEL II (Nihon Abionics) was used for the analysis. A diagram of the system is shown in Figure 1.

The microradiographic image was first placed in the TVIP–4100 television image processor via a videocamera module (XC–77, Sony corp.) attached to the microscope. This apparatus serves mainly for image input, image frame memory (digitalized), and dual value transformation. The processor is connected with a PC–9801 RX (NEC) personal computer which serves as the central processing unit.

To compare this new method with conventional linear analysis, a linear analysis based upon the film density of the microradiograph of the specimen was then carried out using a Sakura Microdensitometer, model PDM–5, Type AR (Konica).
Fig. 2 Color image of a microradiograph of a section from a brown spot on the lingual side of the maxillary second molar of a 36 year old female. By EXCEL II system.

Fig. 4-a: Quantitative contour map constructed by drawing a line connecting each portion with 40, 50, 60 and 70% calcification.

Fig. 4-b: The quantitative contour map is shown using green, blue, red and yellow respectively for 40, 50, 60 and 70%. The decalcified portion under the superficial layer appears to be communicating with the dental surface at the site indicated by the arrow.

Fig. 5-a: The image on the TV monitor is the section immediately mesial to the one shown in Figure 4-a.

Fig. 5-b: Quantitative contour map of the picture shown in 5-a. The decalcified portion under the superficial layer appears to be communicating with the dental surface at the site indicated by the arrow.
**Results**

A color image on the color TV monitor obtained by the input of the microradiographic picture is shown in Figure 2. The system creates an contour map which allows selection among several color images with different color tones representing the degree of calcification of the enamel in the microradiograph.

The density of the image input into the processor is further analyzed into 256 color tones. At any of the color concentrations, dual value transformation of the image can be carried out to represent the degree of decalcification in a stepwise manner.

The degree of calcification of enamel was evaluated based on the degree of darkness in the aluminum reference system of the microradiograph using the formula of Angmar\(^4\) as the volume percent of the mineral (subsequently abbreviated as \(\%\)). By comparing the degree of darkness of the image of the sample with the various degrees of darkness in the

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**Fig. 6-a** : The image on the TV monitor is the section immediately mesial to the one shown in Figure 5-a.

**Fig. 6-b** : Quantitative contour map of the picture shown in Figure 5-a. Lines representing 40, 50 and 60\% overlap each other.

**Fig. 7** A quantitative contour map by conventional linear analysis. Densitometer scanning was done from the dental surface toward the dento-enamel junction at the site indicated by the arrows. The portions representing 40, 50, 60 and 70\% calcification were plotted on the photograph of the microradiograph. A quantitative isodensity linear image was obtained by connecting these points. Scanning was carried out at 50 \(\mu\)m intervals. While the picture as a whole resembles the image shown in figure 4-b, the portion with less than 40\% calcification gives a complex appearance with a discontinuous line of points on the innermost side of the image.
aluminum reference system, it was possible to assign any density to one of the 256 degrees of color in the picture, thus representing the concentration in color. By dual value transformation of the computerized image of the microradiograph, the percentage of calcification was represented on the image seen on the TV monitor. Based on these calculations, the areas where calcification was 40, 50, 60 and 70% were demonstrated stepwise as a quantitative contour map.

Figure 3-a shows the image obtained from dual value transformation of the area of 40% calcification. The image density is separated into an area where mineral content is less than 40% and an area where it is greater than 40%. The area of less than 40% is expressed as white. Based on the results of measurements according to Angmar's formula described above, even the area of greatest calcification in the present specimen corresponded to only 35-40% calcification. This value was subsequently employed as a standard in this study.

Figure 3-b shows considerable irregularity of decalcification through computer generated visual contours of the area.

Similar computation was done at 50, 60 and 70% and the contour lines superimposed to form a single image as shown in Figure 4-a. This type of image is called a quantitative contour map.

Figure 4-b is the same quantitative contour map in a color display. The 70% line appears yellow, the 60% line appears red, the 50% line appears blue and the 40% line appears green.

Figure 5-a is the monitor image of the section immediately mesial to the one shown in figure 4 and 5-b is the quantitative contour map of the same section.

Figure 6-a is the monitor image of the section immediately mesial to the one shown in figure 5 and 6-b is the quantitative contour map of the same section.

Such computer simulations may be easily accomplished within a short time once the image of the specimen has been input into the processor. It is possible to not only represent any percentage desired as an image on the screen but also to know the degree of calcification of any area with a selected density on the image by calculating the transformed dual value.

The quantitative contour map obtained by conventional linear analysis is shown in Figure 7. Measurement of the density was accomplished at 47 sites as indicated by arrows.

This was accomplished by scanning at 50 μm intervals from the dental surface, along the enamel prisms, to the deep enamel layer. Using the formula of Angmar et al., a quantitative contour map was composed from these linear analysis values.

The yellow line in the image represents 70% calcification, the 60% line appears red, the 50% line appears blue.

The portion representing less than 40% is expressed as the area beyond the green line and comprises most of the enamel.

**Discussion**

The quantitative assessment of the degree of calcification at the site of initial carious change in enamel has customarily been accomplished by densitometric measurement of the microradiograph\(^5\)\(^7\). Most of these methods have utilized linear analysis, making it difficult to assess the state of mineral distribution as a plane.

In recent years, attempts have been made to quantify the degree of calcification of the teeth using computerized image analysis\(^8\)\(^\text{--}^\text{10}\). These methods were thought to supplement the limitations of macroscopic observation\(^9\). As yet, the ability to manipulate and analyze the data appears to be unsatisfactory in either of these methods. The picture of the communication between the dental surface and the decalcified portion under the surface layer is poorly demonstrated under normal microscopic observation. This communication is shown by the arrow in figure 4-a and 5-b and is obtained by quantitative observation using dual value transformation. The authors believe that this picture represents the communication between the enamel surface and the decalcified lesion immediately beneath the surface.

In addition to making it possible to represent as a percentage the degree of calcification (or decalcification) represented by a given degree of radiographic density, such methods
of quantitative observation by dual value transformation offer an extremely useful method for advanced studies on the initiation and progress of carious changes. For these purposes, observation under low magnification seems to be necessary so the carious changes can be viewed in their entirety.

Linear analysis by densitometry studies of the microradiograph as described in this study, is very labor intensive. The creation of charts representing linear analysis subsequent to quantitative analysis of the data is indispensable.

As seen in figure 7, a complex mineral distribution greatly increases the difficulty in evaluating of the degree of calcification as a semi-quantitative distribution. In this instance, the appearance of the portion with 40% calcification is so complex that it is not possible to express it accurately and to connect the portions with curves by measuring at 50 μm intervals. Comparison of figure 4-b with figure 7 reveals that while similar images were obtained as a whole, since the methods were identical in principle, that the image in figure 7 is clearly less detailed.

Although it is theoretically possible to solve these problems by narrowing the interval of scanning, the technique described may represent the limit of this linear analytical method in view of the increase of labor required. Consequently, this method does not appear to be suitable for visualization of the deepest portion of the decalcification through visualization of complex mineral distribution.

The method of image analysis employed in the present study makes it possible to readily obtain a semiquantitative contour map after the input of the images and the assigning of the desired colors for each percentage of calcification according to the formula of Angmar et al. A continuous semiquantitative contour map can thus be efficiently obtained by application of this method to serial sections. This process makes it possible to continuous visualize the quantitative stereostructure of the carious changes by serial sections. The end result is that three dimensional representation and analysis of the carious changes may become possible.

Conclusion

Computerized image analysis appears to make a semiquantitative analysis more feasible than does a method using densitometry. The former method appears to be a superior means of analyzing the details of the process of initiation and spread of carious changes. Moreover, progress is made toward the three dimensional analysis of carious changes by serial sections.

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