

Microscopic Features of Enamel and Dentinal Caries Under Confocal Laser Scanning Microscopy (CLSM) and Image Analyzer: Preliminary Experimental Study

*R Saini, MDS, *A S Azmi, DDS, *N B Ghani, BA, *K A M Al-Salihi, Ph.D

*School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

SUMMARY

This study was designed to identify surface and subsurface microscopic changes in different carious lesions by using Confocal Laser Scanning Microscope (CLSM) and Image analyzer (light microscopy). Thirty extracted carious posterior teeth were fixed, embedded and polymerized in plastic fixation medium. The final thin sections (80µm) were stained with H&E and Masson Goldner's Tricome while others were left unstained. Under Confocal, marked differences between control sound enamel and dentin, and carious area of the samples were observed which illustrated that a correlation existed between the zone of autofluorescence, demineralization and carious enamel and dentin. Compared to CLSM, Image Analyzer only produce two-dimensional images but the histopathological changes were better appreciated by using various staining methods.

KEY WORDS:

Dental caries, Confocal microscopy, Autofluorescence, Image analyzer

INTRODUCTION

Dental caries is defined as a localized, post eruptive, pathologic process of external origin involving softening of the hard tissue and proceeding to the formation of a cavity. Localized destruction of the tooth surface is initiated by decalcification of the enamel followed by enzymatic lysis of organic structures leading to cavity formation¹. The bacteria in the biofilm are always metabolically active causing fluctuations in pH. These fluctuations may cause a loss or gain of mineral content from the tooth when the pH is dropping or increasing respectively². The cumulative result from these demineralization and remineralization processes may be a net loss of mineral, leading to dissolution of the dental hard tissue and formation of a caries lesion. There are several types of dental caries depending on their location such as pits and fissures caries, smooth surface caries, root caries and subgingival caries³. The pits and fissures caries is the most important type because it has the highest prevalence and it provides excellent mechanical shelter for microorganisms such as *S. mutans* and other streptococci⁴. According to the same author, smooth surface caries involve the proximal enamel surfaces immediately gingival of the contact area and it is the second most susceptible area to caries. Microscopically, caries begin with the integration of

the enamel prisms after decalcification of the interprismatic substances, events which lead to the accumulation of debris and microorganisms. When the process reaches the dentinoenamel junction it spread laterally and also penetrates the dentin along the dentinal tubules⁵. Confocal Laser Scanning Microscope (CLSM) is a relatively new, non destructive technique, which provides three-dimensional images by means of microscopic topography. Initially CLSM was applied almost exclusively in cell biology⁶. However, more recently, CLSM has found potential applications in dental caries related studies, including investigation into the demineralization and remineralization processes⁷. This study was designed to identify surface and subsurface microscopic changes in different carious lesions by using Confocal Laser Scanning Microscope (CLSM) and Image analyzer (light microscopy).

MATERIALS AND METHODS

This was an experimental preliminary study. Thirty carious extracted teeth were used in this study. Inclusion criteria for teeth selection was posterior permanent (molars and premolars) teeth with clinically evident smooth surface caries and pits and fissure caries. Exclusion criteria for teeth selection included teeth with previous restoration or extensive caries extending to the pulp.

i. Sample processing

The teeth were fixed as a whole in 10% neutral buffered formalin for duration of 10 days. Diamond Band Cutting System (EXAKT) was used to section the teeth longitudinally into two halves. The specimens were dehydrated with 70%, 90% and absolute alcohol for four days. Clearing was done with xylene for 24 hours and then infiltrated with 10%, 30%, 50%, 70%, 90% and 100% Technovit. Each sectioned tooth was then embedded in Technovit 7200 and polymerized for 16 hours (blocking). The blocks were glued to microscope slides (50x100x1.5mm) by using Technovit 4000. Excess resin was then grinded until the surface of the sample was exposed. The free grinded side was then glued to another smaller microscope slide (25x75x1.5mm) by using Technovit 7210 (Sandwich technique). The 'sandwich' block was polymerized for 15 minutes. The 'sandwich' block was fixed to a vacuum machine and sectioned by the Diamond Band Cutting System (EXAKT) with the thickness of 150-200µm. The slide was further grinded on a grinding machine (EXAKT), until 80µm

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Corresponding Author: Rajan Saini, School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

by using sandpaper sized p350 to p1000. The slides were polished using polishing oil and were inserted into an ultrasonic cleaner for about 15 to 20 minutes. They were then kept overnight for drying. Some of the slides were stained with H&E and Masson Goldner's Tricome while others were left unstained.

ii. Viewing of Slides

The slides were viewed under CLSM (Leica TCS SP 2) and Image Analyzer (Zeiss KS300 Image Analyzing System). Only unstained sections were viewed under Confocal Microscope. Adjacent sound enamel and dentin were used as control references. The carious lesions were scanned after being brought into focus with HCPL Fluotar 10x/0.30 objective and 10x eyepiece. The specimens were illuminated by using a 514-633 nm excitation wavelength. Series of sections were done for each image. By xyz mode scanning of samples at various focal planes along the Z-axis, a three dimensional data is acquired for samples using three dimensional reconstruction software. The images were analyzed using a profile program for lesion depth and image area.

RESULTS AND DISCUSSION

The images showed noticeable differences between control sound enamel and dentin, and carious areas of the samples.

The demineralized areas appeared darker compared to the sound areas. All examined teeth showed varying degrees of histopathological changes from initial lesion to dentinoenamel lesion.

In all the teeth examined under Confocal Laser Scanning Microscopy, sound enamel and dentin did not autofluorescence. Autofluorescence distribution from carious area correlated with the extent of demineralization in the tissue. The marked differences between control sound enamel and dentin, and carious area of the samples illustrated that a correlation existed between the zone of autofluorescence, demineralization and carious dentine that was markedly softened by the carious process. This finding was compatible with those of the previous studies⁸⁻¹⁰. Advantage of confocal microscopy is to make better contrast images due to the advance confocal software used to editing the images which enabled us to label the different area of demineralization with different colour without pre-staining procedures of the sections. Figure 1 shows confocal laser scanning microscope images of smooth surface caries in colour contrast. Sound enamel was seen as green while carious lesion showed red colour. This facility made the identification of the four initial carious zones easily detectable. Confocal microscopy provided three-dimensional views for carious lesion. Images of autofluorescence carious

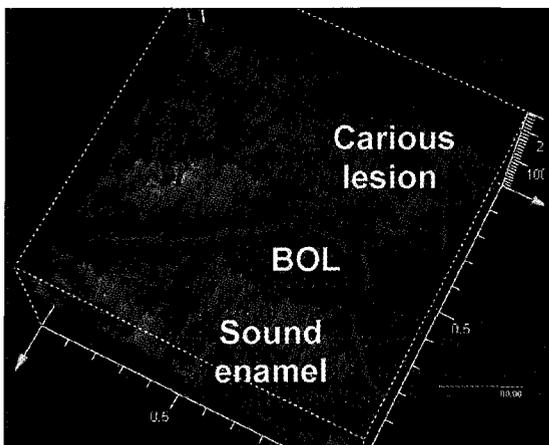


Fig. 1: Initial pits and fissures caries in colour contrast.

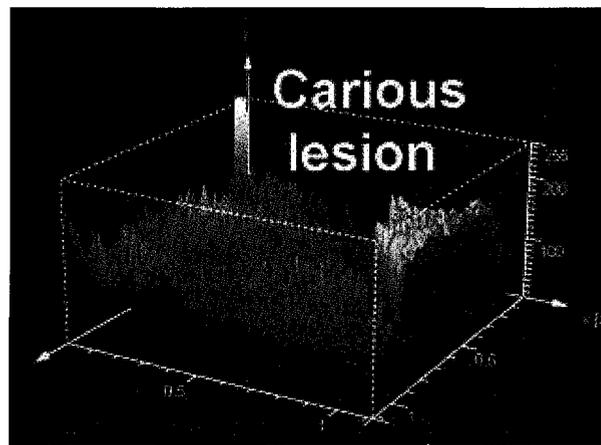


Fig. 2: CLSM 3-D and topography image reveals the distribution of the carious lesion.

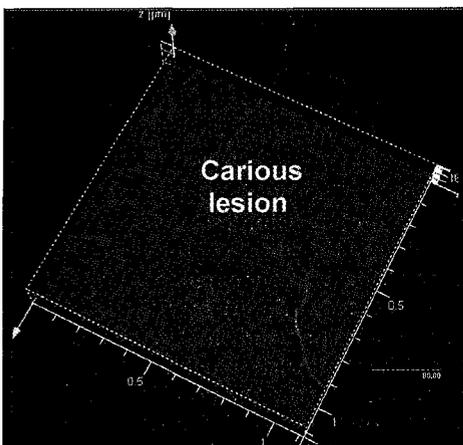


Fig. 3: Photomicrograph of confocal microscopy image reveals the topographical appearance of initial carious lesion.

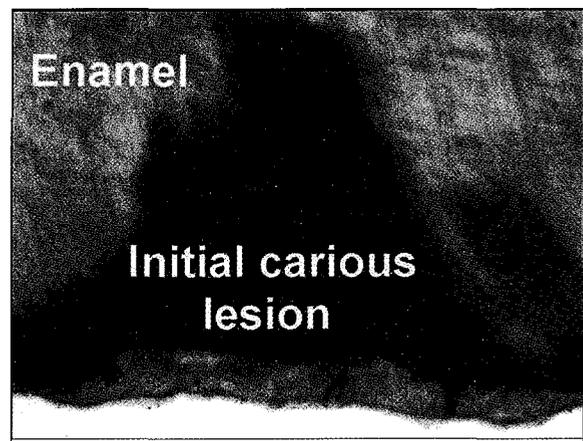


Fig. 4: Initial Smooth Surface Caries involving the enamel with H&E staining. (10x)

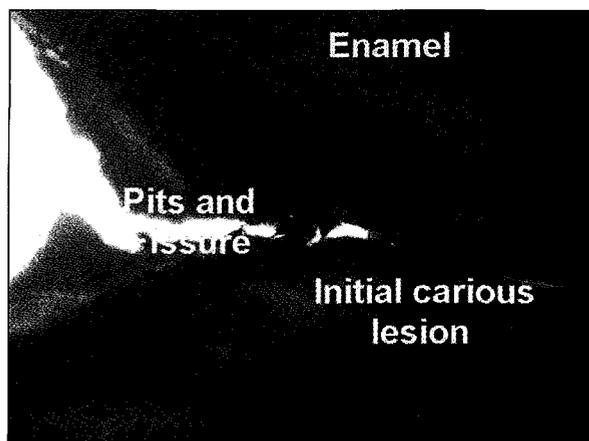


Fig. 5: Pits and fissures caries with Masson Goldner's Tricome staining.. (10x)

area were viewed in 3D projection planes xy, xz and yz. This enabled us to determine the demineralization and remineralization process and the depth of the lesions. The 3-D images of carious lesions appeared as curved, straight or combination of both depending on the nature of the lesion (Fig.2). Figure 3 shows the photomicrograph of confocal microscopy image revealing the topographical appearance of initial carious lesion which is visible as the triangular elevated area. Under Image Analyzer, the initial carious lesion of a smooth surface caries revealed a conical shape with its apex towards the dentin. The various zones of differing translucencies were enhanced in specimens with H& E (Fig.4) and Masson Goldner's Tricome (Fig.5) staining. The deep advancing edge of the lesion revealed a translucent zone (first), the dark zone (second), the body of the lesion (third) and the surface zone (fourth). Other teeth, which involved dentinoenamel caries, showed extension of the lesions along the dentinoenamel junction and lateral spread. These findings were compatible with previous studies on histopathological changes in dental caries^{7, 11, 12}.

Image analyzer can only produce two-dimensional images. In contrast, confocal microscope images can be manipulated for topography functions and 3-D image reconstruction can be performed. Analysis of 3-D images by the use of topography function maps the real surface structure of the carious lesion.

CONCLUSION

Viewing of teeth by CLSM has shown that a correlation existed between the zone of autofluorescence, demineralization and carious lesion that was markedly softened by the carious process. CLSM offers a unique, sensitive and valid method for qualifying and quantifying mineral loss in enamel and dentine carious lesion without any tissue damage. The histopathological changes of different caries process can be appreciated better under light microscopy image analyzer by using various staining methods. This study demonstrates the possibility of using both CLSM and Image Analyzer in understanding the histopathological structural changes in tooth associated with carious lesions in addition to determining demineralization and remineralization changes.

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