Histological Aspects of Human Enamel Fissure Caries studied by CLSM

T. Nishikawa¹, S. Yoshida¹, A. Tanaka¹, H. Zoellner ² and D. M. Walker ²
1. Dept of Oral Pathology, Osaka Dental University, Japan. 2. Dept of Oral Pathology, Faculty of Dentistry, Univ of Sydney, Australia

INTRODUCTION
The lesions found in dental tissues in dental caries have been examined by light or electron microscopic methods using decalcified or ground sections. No three dimensional observations of carious teeth have been performed, since thin sections are difficult to prepare serially, due to the low organic content which is approximately 1% of the enamel content. Examinations using confocal laser scanning microscopy (CLSM) have been described in deep portions of hard tissue without actually destroying the overlying tissues yielding tomographic images of in-vitro microbially secondary caries [1] and the interface between enamel and dentinal restorations [2,3]. There are however no corresponding CLSM studies of dental caries or comparisons with the changes seen by other established techniques. This study dealt with comparative findings obtained from light microscopy, microradiography and CLSM in an early lesions of human enamel caries.

MATERIALS AND METHODS
100 µm ground sections from permanent human teeth (n=38; premolars n=5 and molars n=33) with early fissure enamel caries were stained with 0.5% acid fuchsin in propylene glycol for 5 minutes and dehydrated in graded alcohols and xylol, and embedded in mounting medium (Eukitt, refractive index 1.5, O.Kindler GmbH & Co., Freiburg, Germany). The 38 specimens were brought into contact with an ultra fine-grain film (Kodak spectroscopic safety film type No. 649-0) and irradiated with soft x-rays for 2 minutes using a cabinet soft X-ray apparatus (Sofron SRO-M50, Soken, Japan) to obtain contact microradiographs. These specimens were also observed by transmitted light microscopy, and via tomographic images taken at levels of increasing depth of either 2 or 5 µm from the section surface, using an Olympus LSM-G200 confocal laser scanning microscope with an argon ion laser radiation source, followed by image processing via a Hewlett Packard, Vectra 386/25 computer to obtain image analysis of the fluorescence intensity gradient. The argon laser excitation wavelength was 488 nm (λ exc). The lesions were radio-opaque, the body of the lesion and the dark zones were yellow with the 535 nm and 590 nm filters simultaneously. The translucent zones displayed dark green fluorescence with the 535 nm filter alone and green or yellow fluorescence with the 535 nm and 590 nm filters together (Fig 3).

The CLSM image analysis of carious lesions observed with the 590 nm filter alone was performed to assess colour-graded fluorescence intensity. The lesions progressed along the peripheral portion of the enamel rod-like structures, with strong intensity in the surface zone. In the surface side of the body of the lesion, the prismatic structure of the enamel rods and the carious lesions from the tooth surface to the dentino-enamel junction were apparent at some peripheral portions of the enamel rods by CLSM. The enamel rods, with carious cones having bases on the dentine sides. These carious lesions had a series of zones from outside to inside: surface zone, body of the lesion, dark zone, and translucent zone [4] (Fig 1).

Microangiography
The surface zone and the translucent zone were radio-opaque, the body of the lesion and the dark zone showed various degree of radio-lucency and accentuation of the striae of Retzius existed in the radiolucent areas (Fig 2).

CLSM
All enamel caries were brighter than normal enamel, which did not fluoresce, whether the 535 nm (λ em) filter (green) or 590 nm (λ em) filter (red) was used. The area of altered enamel identified by CLSM was more clearly identified than that apparent by light microscopy or microangiography. The surface zone had yellow fluorescence with the 535 nm and 590 nm filter together. The surface side of the bodies of the lesions had red fluorescence with the 590 nm filter but appeared black viewed with the 535 nm filter; the striae of Retzius and the dark zones were yellow with the 535 nm and 590 nm filters simultaneously. The translucent zones displayed dark green fluorescence with the 535 nm filter alone and green or yellow fluorescence with the 535 nm and 590 nm filters together (Fig 3).

The CLSM image analysis of carious lesions observed with the 590 nm filter alone was performed to assess colour-graded fluorescence intensity. The lesions progressed along the peripheral portion of the enamel rod-like structures, with strong intensity in the surface zone. In the surface side of the body of the lesion, the prismatic structure of the enamel was lost (Fig 4).

Tomographic images taken at 5 µm depth intervals by CLSM at this site demonstrated that the lesions were approximately spheroid instead of flare-shaped (twin cone-shaped) as seen by other techniques (Figs 5 and 6) [5].

Changes in enamel and rod structure in the body of the lesion
At high magnification CLSM with image analysis using the 590 nm filter alone, intense fluorescence was seen in almost the entire body of the lesion. The outside of the body of the lesions indicated followed two fluorescent patterns: one in which fluorescence was present along the central portion of the enamel rods, and the second at their periphery (Fig 7).

Observation of enamel lamella-like structures, aprismatic zones
Enamel lamella-like structures extending from the tooth surface to the dentino-enamel junction were apparent at some peripheral portions of the enamel rods by CLSM. The enamel rods, with carious cones having bases on the dentine sides. These carious lesions had a series of zones from outside to inside: surface zone, body of the lesion, dark zone, and translucent zone [4] (Fig 1).

Microscopy and Analysis • January 2003

prisms adjacent to the lamella-like structures had fluorescence at their peripheries and cross-striations, as seen by CLSM using the 535 and 590 nm filters. The enamel region adjacent to the dentine had a non-fluorescent and aprismatic zone 15-20 µm in thickness. Dentine immediately below the affected enamel had fluorescence along some individual tubules (Fig 8).

**DISCUSSION**

CLSM is characterised by the advantage of confocal imaging, enabling observation of plane images at varying depths in samples without slicing the specimen, the scanning plane thickness being 0.1 µm. CLSM is unique in that not the surface but deep portions of the specimen can be observed at high resolution without mechanical disruption of the tissue. CLSM avoids the interference produced by section artifacts by observing only deep structure from the section surface. Specimen thickness does not matter; it might be possible to recognise portions not affected by acid treatment for decalcification or mechanical damage during ground sectioning and also tomographic images could be detected at microscopic levels [6].

CLSM requires that the specimen produces fluorescence. Since normal enamel does not autofluoresce, it is difficult to observe by CLSM. Acid fuchsin, which used as a clinical guide for caries detection, stains plaque and carious enamel [7]. And enamel caries was detected under the condition of 530 nm (λ exc) and 587 nm (λ em), or 400 nm (λ exc) and 510 nm (λ em) [8]. In the present study, sufficient fluorescence to show early changes in the striae of Retzius [9] and cross-striations [10] in carious enamel were apparent with 488 nm (λ exc) and 590 nm (λ em) filters of CLSM. With the use of the 535 nm (λ em) filter, enamel caries could been seen with or without acid fuchsin staining.

A study which showed dentine and enamel caries under the condition of 488 nm (λ exc) and 540 nm (λ em) [11] suggested that demineralization and the import of exogenous fluorescent molecules during carious process etc. seem to cause the mechanism, though it is not yet well-known. Enamel rods consist of a central and a peripheral portion, according to differences in crystal orientation. The scanning electron microscopy of an acid-etched transverse enamel section showed that the central or the peripheral portions appear to resist etching [12,13]. Using CLSM, there were some differences to identify the zones in the lesions corresponding to those observed by conventional techniques such as light microscopy, microradiography, polarizing microscopy and electron microscopy. Unique CLSM imaging technology, in contrast to conventional techniques as previously used, needs further refinements as a means of observing caries, particularly the investigation of other recommended fluorescent dyes such as rhodamine and Villanueva bone stain [14,15].

**CONCLUSIONS**

CLSM observation for early enamel caries is characterised by a comparatively non-destructive investigation that yields tomographic images corresponding in part to the histological images obtained by previously established techniques, and findings not visualised by previous techniques, suggesting that CLSM is a useful tool available for the detection of new caries.
Carious enamel is more extensive under the CLSM findings than suggested by either transmitted or polarised light microscopy, and the caries lesion in CLSM often exhibits different zones to those obtained by earlier techniques or microradiography. CLSM shows clearly the pattern of carious attack occurring at the central or peripheral portion of the enamel rods at the advancing front of the lesion, presumably reflecting susceptible sites of demineralisation by plaque acid. The altered fluorescence shown in the enamel-lamella like structures and adjacent enamel and dentine confirms that demineralisation might proceed preferentially via these structures. CLSM also confirms that an aprismatic layer exists at the dentino-enamel junction which appears to be caries-resistant.

REFERENCES