Lasers in the Life Sciences, Vol. 8, pp. 1–12
Reprints available directly from the publisher
Photocopying permitted by license only

Effects of CO_2 Laser on Human Dentin: A Confocal Laser Scanning Microscopic Study

YUICHI KIMURA [a,*], PETRA WILDER-SMITH [b], TATIANA B. KRASIEVA [b], LIH-HUEI L. LIAW [b] and KOUKICHI MATSUMOTO [a]

[a] *Department of Endodontics, Faculty of Dentistry, Showa University, 2-1-1, Kitasenzoku, Ohta-ku, Tokyo 145, Japan;* [b] *Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Sciences Road East, Irvine, CA 92715, USA*

(Received 18 December 1996)

In this study, the effects of dentin ablation using a CO_2 laser at 10.6 μm were visualized using a fluorescence technique and confocal laser scanning microscopy. Thirty extracted human teeth showing no clinical signs of caries were investigated. All teeth were horizontally sectioned to approx. 200 μm thickness and sections were irradiated at different parameters as follows: 3 W [0.01 s pulse duration (p.d.)], 4 W (0.01 s p.d.), and 0.3 W (0.1 s p.d.) using CO_2 laser. After laser irradiation, samples were treated with sodium hypochlorite, stained using Rhodamine 123, and observed with confocal laser scanning microscopy followed scanning electron microscope procedure. Surface images obtained using confocal laser scanning microscope were similar to those observed with scanning electron microscopy, but subsurface imaging to a depth of approx. 60 μm which were different from surface ones was achieved using confocal laser microscope techniques. Small effects of laser irradiation were also observable using CLSM easily. This fluorescence technique offers a useful new alternative for visualization and quantification of laser-induced dentin ablation.

Keywords: Dentin tubules; Laser dental ablation; Fluorescent dye; Tooth ablation; Rhodamine 123; CO_2 laser; Confocal microscopy; Fluorescent microscopy

* Corresponding author. Tel.: 81-3-3787-1151(ext. 247). Fax: 81-3-3787-1229. E-mail: yukimura@hp.dent.showa-u.ac.jp.

INTRODUCTION

Several papers have been published concerning the changes in dentin resulting from irradiated with the CO_2 laser (Cooper *et al.*, 1988; Kantola, 1973; Nelson *et al.*, 1986a). In sound dentin, the high absorption of the CO_2 laser irradiation gives rise to a temperature increase which, after evaporation of water, causes the combustion of the organic material and the fusion of the hydroxyapatite (Nelson *et al.*, 1986b). Early investigations revealed that this irradiated dentin recrystallizes while cooling to form a structure similar to that of normal enamel (Kantola, 1973). Little is known about thickness or characteristics of the surface melt zone. The sealing effect of the laser may be of interest in rendering dentin less susceptible to demineralization (Nammour *et al.*, 1992).

Since the development of a scanning confocal optical system for the microscope in 1957, this device has been extensively modified and applied in many fields (Inoue, 1990). In the dental field, scanning confocal microscopy has been used for a wide range of applications (Kodaka *et al.*, 1993; Torabinejad *et al.*, 1993; Watson, 1989; 1990a; 1990b; 1991a; Watson *et al.*, 1991; 1992; Watson and Wilmot, 1992).

Confocal laser scanning microscope (CLSM) systems can scan a laser beam over stationary samples (Watson, 1991b). Surface images obtained using the confocal microscope are similar in character to those provided by scanning electron microscopy (SEM), but subsurface imaging is also possible. Furthermore, confocal microscope images can be three dimensionally reconstructed (Carlsson *et al.*, 1985; Watson, 1991b). Conventional dehydration and gold-coating methods utilized for SEM have several disadvantages when compared to sample preparation for CLSM: dehydration and heating can damage specimens and cause a variety of artifacts such as cracking or "bubbling". Tooth samples can be visualized using the confocal laser scanning microscope either by lightly metal-coating the surface, or by using fluorescence techniques.

A variety of dyes and fluorescence staining techniques have been developed to achieve maximum imaging depth and resolution in healthy dentin (Kimura *et al.*, 1996; Watson, 1990a; 1994).

The purposes of this study were to investigate the surface and subsurface changes in dentin after ablation using the CO_2 laser,

and to compare observations obtained using CLSM and SEM techniques.

MATERIALS AND METHODS

Sample Preparation

Thirty extracted human teeth showing no clinical signs of caries, stored in demineralized water with 0.01% (w/v) thymol, were horizontally sectioned into thin slices (approx. 200 μm thickness) using a low speed saw with coolant (Isomet, Buehler, IL, USA).

Laser Device and Irradiation

This study was performed using a XANAR CO_2 laser XA-50 System (Johnson & Johnson Company, USA). This laser emitted at a wavelength of 10.6 μm and used an articulated arm delivery system with a focusing handpiece. The parameters used were 40 mJ/pulse [4 W, 0.01 s p.d., energy density (ED) 41.6 J/cm^2], 30 mJ/pulse (3 W, 0.01 s p.d., ED 31.2 J/cm^2) and 30 mJ/pulse (0.3 W, 0.1 s p.d., ED 31.2 J/cm^2), and the spot size measured 0.35 mm. All samples were clamped during laser treatment and exposed to one pulse only of laser irradiation.

Staining Procedure

After pretreatment with sodium hypochlorite (NaOCl) (5.25% by Wt, Darrow Comp., CA, USA) for one hour under vacuum and ultrasonication, samples were stained using Rhodamine 123 (Eastman Kodak CO., NY, USA) at a concentration of 10^{-5} M in phosphate buffer saline (PBS) for one hour under vacuum and ultrasonication (Kimura *et al.*, 1996). After staining, sections were washed 2–3 times with PBS, blotted and fixed to slide glasses with cyanoacrylate glue.

Confocal Laser Microscope Device

Stained samples were examined using an LSM 410 inverted Zeiss laser scanning microscope (Carl Zeiss, Oberkochen, Germany). Stacks of thin optical sections were obtained for each sample. The objective lens used was the Plan-Neofluar 100× bright field, n.a.1.3, oil

immersion (Carl Zeiss, Oberkochen, Germany). The laser wavelength of 488 nm was used for fluorescence excitation; emission was isolated with a long pass 520 nm filter. The distance between optical sections was 2 or 4 μm on the Z-axis. Overall depth of acquisition ranged from approx. 30 μm to 116 μm depending on depth of penetration of Rhodamine 123 into the sample. The information obtained was stored on 1 GByte optical disc (Panasonic, Japan) and 3-dimensional images were generated from stacks of stored images using original LSM 410 software.

SEM

SEM was performed to identify the surface structural effects of laser irradiation on the dentin surface. After observation using CLSM, the samples were dehydrated in a graded series of aqueous ethanol (30, 50, 70, 90, and 100% ethanol) for 10 minutes at each concentration, mounted on stubs using colloidal silver liquid (Ted Pella, CA, USA) and gold coated on a PAC-1 Pelco advanced coater 9500 (Ted Pella, CA, USA). Micrographs of the dentin surface were taken on a Philips 515 (Mohawk, NJ, USA) SEM.

RESULTS

Figures 1(a,b) show surface effects seen by SEM after irradiation at 3 W (0.01 s p.d.). The residual surfaces showed ablation and melting, but some dentinal tubules appeared patent (a,b). Figures 2(a,b) depict surface effects viewed by SEM after irradiation at parameter 4 W (0.01 s p.d.). The surfaces of irradiated areas were ablated and melted, but some dentinal tubules in these spots were still open (a,b), with a similar appearance as in Fig. 1. Additionally, a white marginal frosted zone was evident around the periphery of the irradiated area (a), which was barely recognizable in Fig. 1(a). Cracks in the samples irradiated at 4 W was more extensive than in samples irradiated at 3 W. Figures 3(a,b,c,d) show the CLSM images of samples irradiated at 0.3 W (0.1 s p.d.). The changes in the marginal frosted zone and irradiated area were observed easily at low magnification using CLSM techniques (a,b). On the surface, no ablation,

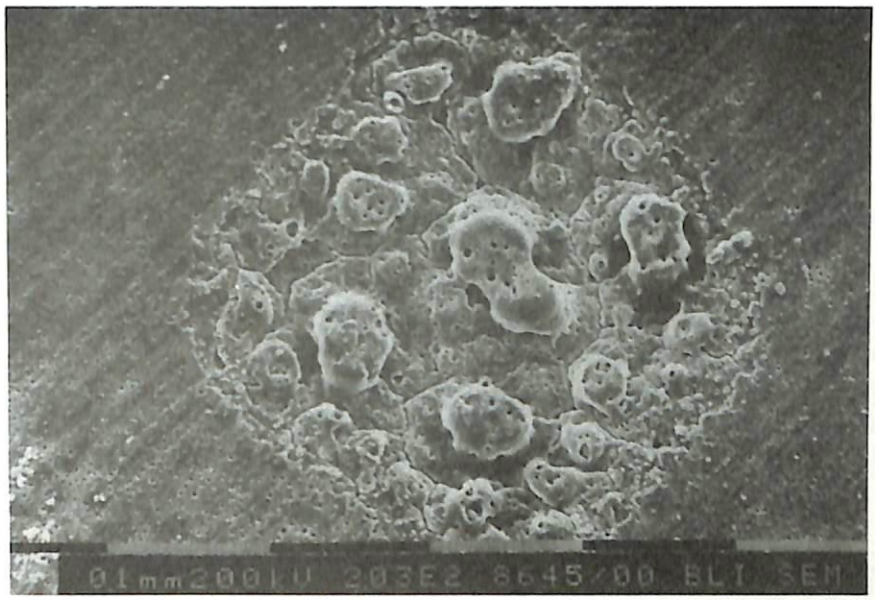

FIGURE 1(a)

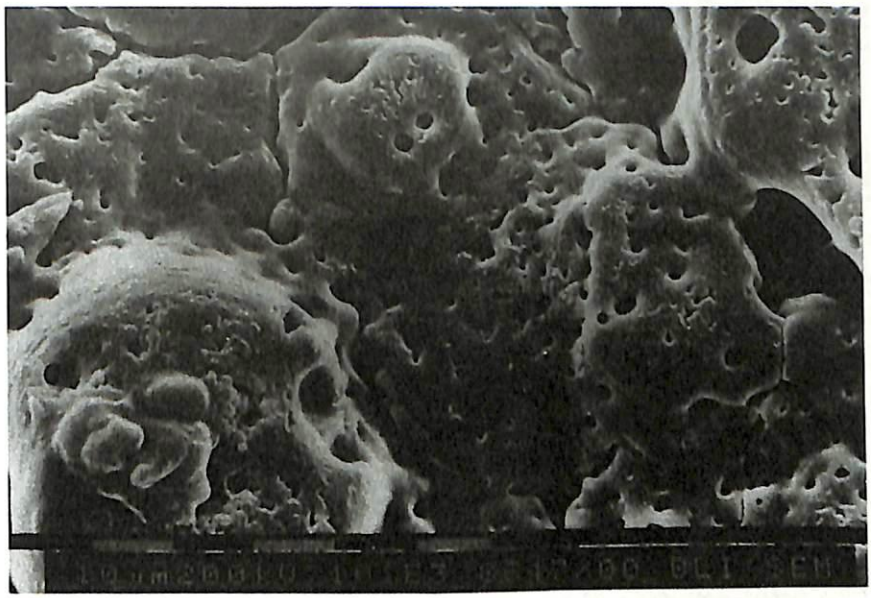

FIGURE 1 SEM photographs of samples irradiated at 3 W (0.01 s p.d.). (a) at a magnification of $\times$ 170, and (b) at a magnification of $\times$ 1010. The scale represents 0.1 mm (a), and 10 μm (b).

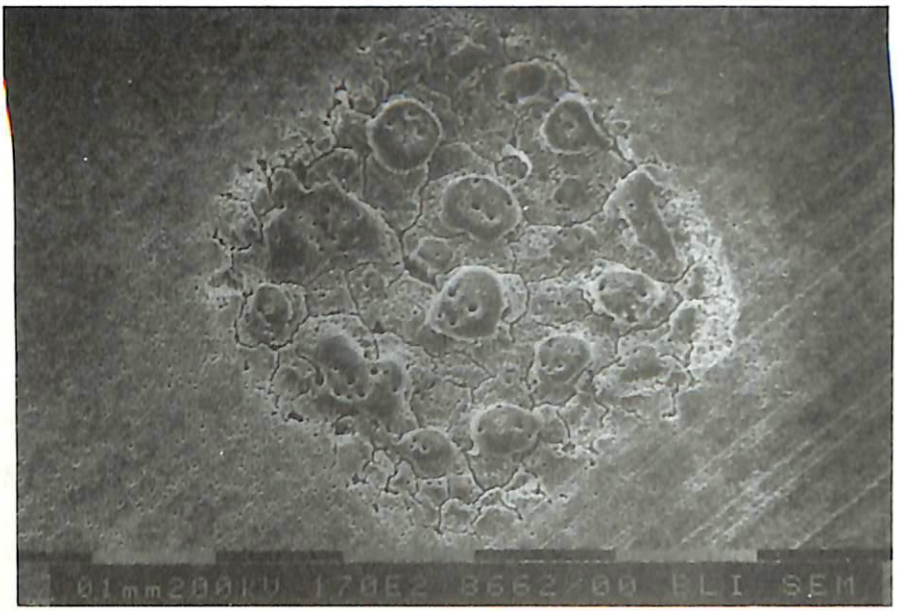

FIGURE 2(a)

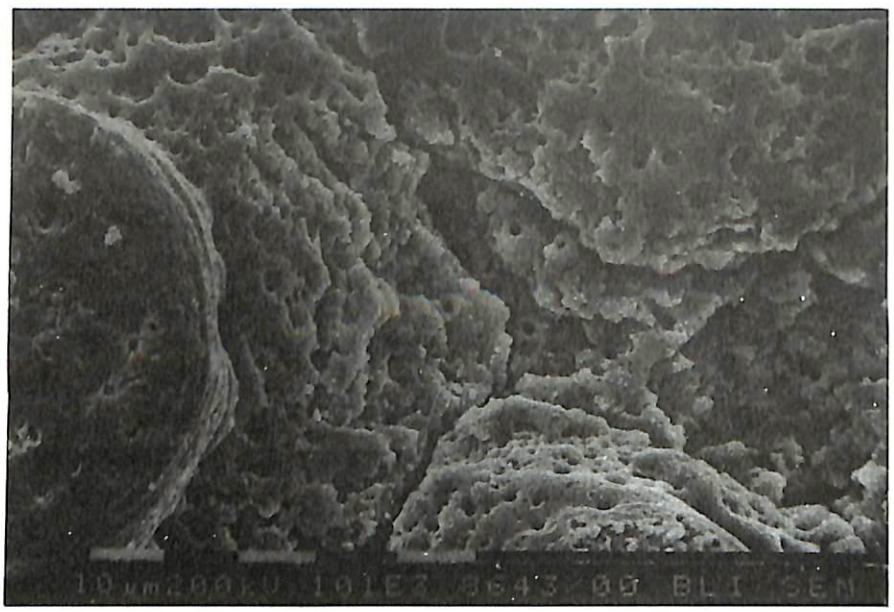

FIGURE 2 SEM photographs of samples irradiated at 4 W (0.01 s p.d.). (a) at a magnification of $\times 203$, and (b) at a magnification of $\times 1010$. The scale represents 0.1 mm (a), and 10 μm (b).

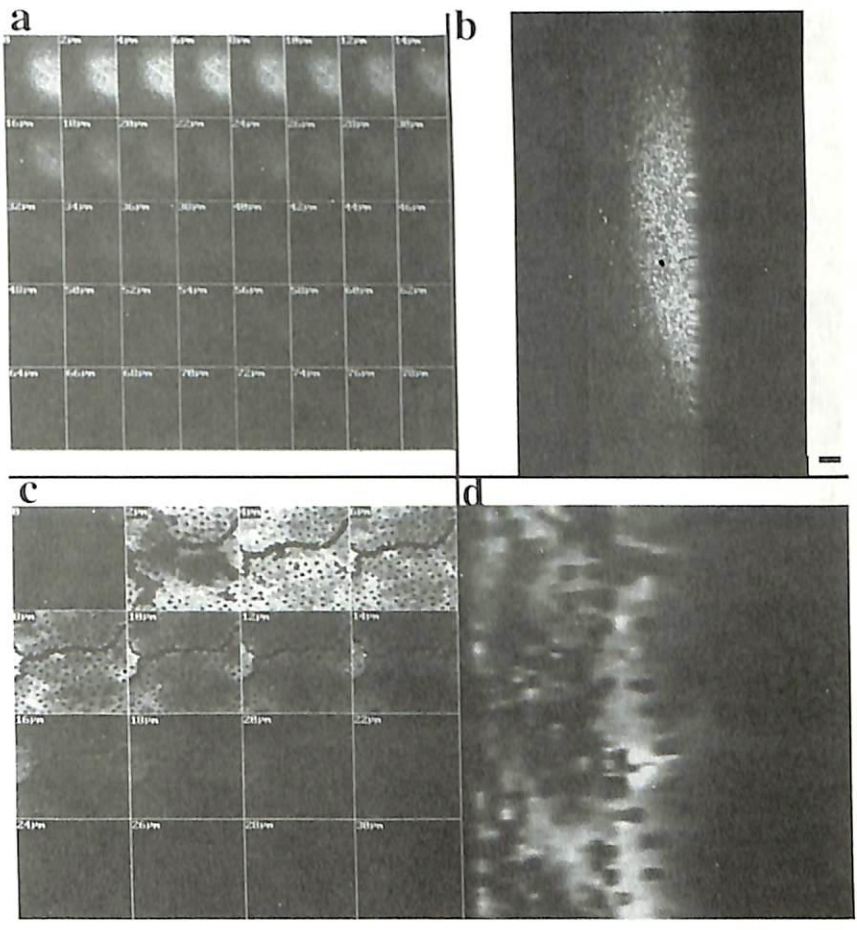

FIGURE 3 CLSM images of samples irradiated at 0.3 W (0.1 s p.d.). (a,b) at a magnification of × 100, and (c,d) at a magnification of × 1000. (a) is a series of 4 μm sections, (b) is the 3-D lateral view (angle 70°), (c) is a series of 2 μm sections, and (d) is the 3-D lateral view (angle 70°). The scale represents 50 μm (b) or 5 μm (d).

melting, or closure of dentinal tubules was apparent in this area. In a 3-D lateral view (b), dye penetration in the irradiated spot was almost same as other non-irradiated area, but small crack-like lesions were observed and areas of localized staining appeared around the dentinal tubules at 2–8 μm depth at high magnification (c). Figures 4(a,b,c,d) show CLSM images of samples irradiated at 3 W (0.01 s p.d.). On the surface, a few dentinal tubules in the irradiated area appeared open, but subsurface most dentinal tubules were closed at (c,d), and dye penetration in these areas was poor (a,b) (within 10 μm) compared

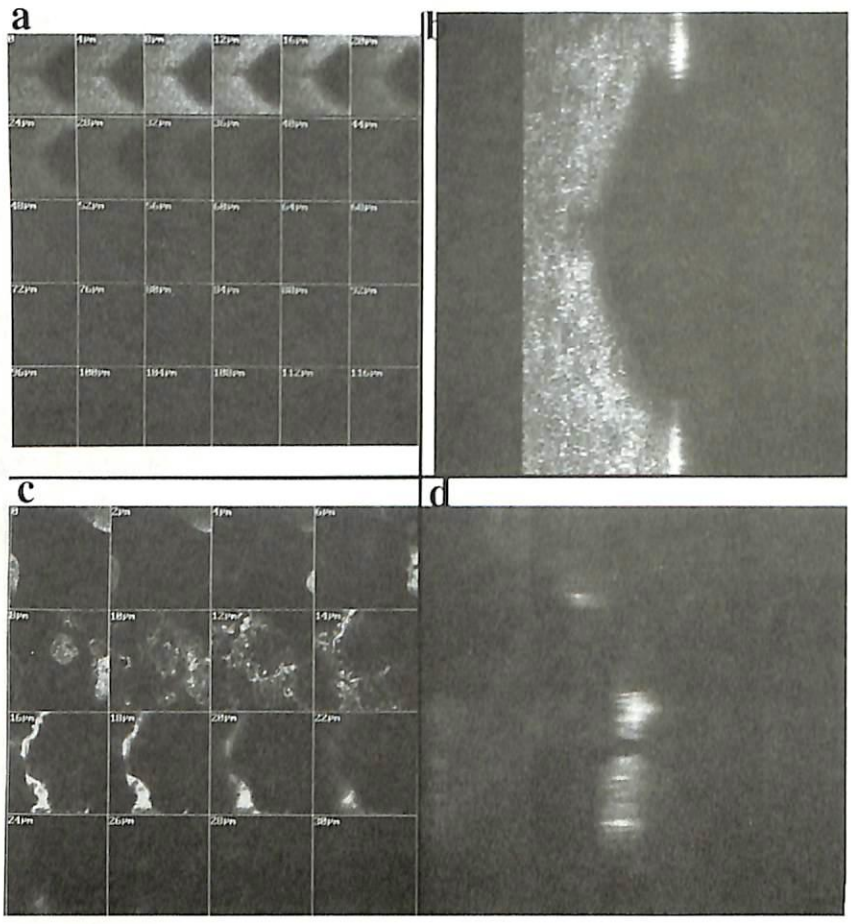

FIGURE 4 CLSM images of samples irradiated at 3 W (0.01 s p.d.). (a,b) at a magnification of × 100, and (c,d) at a magnification of × 1000. (a) is a series of 4 μm sections, (b) is the 3-D lateral view (angle 70°), (c) is a series of 2 μm sections, and (d) is the 3-D lateral view (angle 70°). The scale represents 50 μm (b) or 5 μm (d).

with non-irradiated area (within 60 μm). The marginal frosted zone which was barely visible in SEM photographs was recognized distinctly in the CLSM images (a,b). However, most of dentinal tubules in this zone were still open (b), but dye penetration into this zone was less than that into the non-irradiated area. Figures 5(a,b,c,d) show CLSM images of samples irradiated at 4 W (0.01 s p.d.). The white marginal frosted zone in CLSM images (a,b) was more marked than in Figs. 4(a,b), or in Fig. 2(a). Dye penetration into the irradiated area was poorer than in Fig. 3, or Fig. 4.

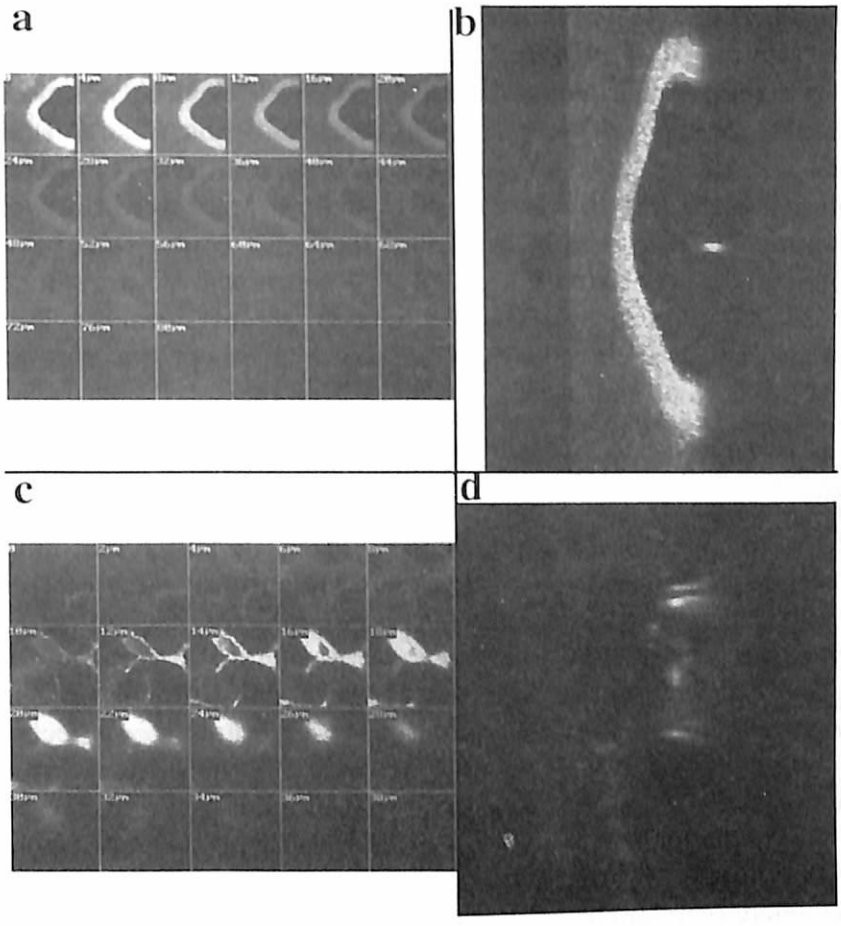

FIGURE 5 CLSM images of samples irradiated at 4 W (0.01 s p.d.). (a,b) at a magnification of × 100, and (c,d) at a magnification of × 1000. (a) is a series of 4 μm sections, (b) is a 3-D lateral views (angle 70°), (c) is a series of 2 μm sections, and (d) is a 3-D lateral view (angle 70°). The scale represents 50 μm (b) or 5 μm (d).

DISCUSSION

In this study, the surface and subsurface effects of laser-induced ablation of dentin were investigated using SEM and CLSM. In this investigation dentinal tubules on the surface appeared patent using SEM techniques. However, according to Nammour *et al.* (1992), most surface dentinal tubules were sealed after CO_2 laser irradiation. Perhaps these differences can be attributed to the far low energy

densities and single laser pulses used in our investigation, which will have generated lower temperatures than the higher parameters investigated by Nammour. However, in our samples, dentinal tubules in the subsurface layers appeared closed or sealed using CLSM techniques. It might be due to subsurface heat accumulation. The thermal diffusion of the CO_2 laser in enamel is such that one would expect a temperature rise just below the surface (approx. 10 μm depth) (Stern *et al.*, 1972). Laser-induced ablation at 4 W seemed almost same as that at 3 W as to recrystallized dentin, amount of surface ablation and morphological effect by SEM photographs except that the marginal frosted zone and cracks were more pronounced. Similar results were observed by CLSM.

Compared with SEM, the magnification provided by confocal microscopy is low (maximum × 1000). However, within this range, CLSM allows surface and subsurface visualization in three dimensions using a fluorescent dye. Dimensional quantification is easy and accurate: thus we were able to follow and measure the course and dimensions of dentin tubules easily using marker systems on the computer screen (Watson, 1991b). Our investigations of fluorescence staining techniques for ablated dentin showed that laser effects in the subsurface layers were very different from those on the surface, which resembled closely those observed using SEM photographs. The white marginal frosted zone around the irradiated spot which was not readily apparent using SEM was easily observed by CLSM and fluorescent staining. This white zone might be related to its crystal structure. Further study about this will be needed.

Disadvantages of CLSM techniques include its lack of suitability for clinical investigations, as relatively thin sample sections are needed. However, the high-frame speed of the TSM enables real time examination of teeth *in vivo* (New *et al.*, 1991; Watson, 1994). In conclusion, the surface and subsurface visualization of laser ablated dentin was achieved in this study. This technique promises substantial improvements in our capacities to observe laser effects in hard tissues.

Acknowledgements

This study was supported by DOE grant DE903-91ER61227, ONR grant N00014-90-0-0029, NIH grant RR01192 and NIH grant

CA-62203. It was made possible, in part, through access to the Laser Microbeam and Medical Program (LAMMP) and the Clinical Cancer Center Optical Biology Shared Resource at the University of California, Irvine. We would like to thank Dr. Michael W. Berns for his advice and support.

We are also indebted to Dr. Anna Marie A. Arrastia-Jitosho, Mr. A. Nguyen and Mr. B. Tran for their assistance in this study.

References

Carlsson, K., Danielsson, P. E., Lenz, R., Liljeborg, A., Majlof, L., and Aslund, N., Three-dimensional microscopy using a confocal laser scanning microscope. *Optics Letters* **10**(2): 53–55; 1985.

Cooper, L. F., Myers, M. L., Nelson, D. G., and Mowery, A. S., Shear strength of composite bonded to laser-pretreated dentine. *J. Prosthet. Dent.* **60**: 45–49; 1988.

Inoue, S., Foundations of confocal scanned imaging in light microscopy, pp. 1–14. In: *"Handbook of biological confocal microscopy"* (Ed. J. B. Pawley), Plenum Press, NY and London, 1990.

Kantola, S., Laser induced effects on tooth structure. VII. X-ray diffraction study of dentine exposed to a CO_2 laser. *Acta Odontol. Scand.* **31**: 381–386; 1973.

Kimura, Y., Wilder-Smith, P., Krasieva, T. B., Arrastia-Jitosho, A. M. A., Liaw, L-H. L., Matsumoto, K., and Berns, M. W., Visualization and quantification of healthy and carious dentin structure using confocal laser scanning microscopy. *SPIE proceedings* **2672**: 64–72; 1996.

Kodaka, T., Kuroiwa, M., and Kobori, M., Scanning laser microscopic surface profiles of human enamel and dentin after brushing with abrasive dentifrice *in vitro*. *Scanning Microsc.* **7**: 245–254; 1993.

Nammour, S., Renneboog-Squilbin, C., and Nyssen-Behets, C., Increased resistance to artificial caries-like lesions in dentin treated with CO_2 laser. *Caries Res.* **26**: 170–175; 1992.

Nelson, D. G. A., Jongebloed, W. L., and Featherstone, J. D. B., Laser irradiation of human dental enamel and dentine. *NZ Dent. J.* **82**: 74–77; 1986a.

Nelson, D. G. A., Shariathi, M., Glena, R., Shields, C. P., and Featherstone, J. D. B., Effect of pulsed low-energy infrared laser irradiation on artificial caries-like lesion formation. *Caries Res.* **20**: 289–299; 1986b.

New, K. C., Petroll, W. M., Boyde, A., Martin, L., Corcuff, P., Leveque, J. L., Lemp, M. A., Cavanagh, H. D., and Jester, J. V., *In vivo* imaging of human teeth and skin using real-time confocal microscopy. *Scanning* **13**: 369–372; 1991.

Torabinejad, M., Watson, T. F., and Pitt Ford, T. R., Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J. Endodon.* **19**(12): 591–595; 1993.

Stern, R. H., Vahl, J., and Sognnaes, R. F., Lased enamel: Ultrastructural observations of pulsed carbon dioxide laser effect. *J. Dent. Res.* **51**(2): 455–460; 1972.

Watson, T. F., A confocal optical microscope study of the morphology of the tooth/restoration interface using Scotchbond 2 dentin adhesive. *J. Dent. Res.* **68**(6): 1124–1131; 1989.

Watson, T. F., A confocal microscopic study of some factors affecting the adaptation of a light-cured glass ionomer to tooth tissue. *J. Dent. Res.* **69**(8): 1531–1538; 1990a.

Watson, T. F., The application of real-time confocal microscopy to the study of high-speed dental-bur-tooth-cutting interactions. *J. Microsc.* **157**(1): 51–60; 1990b.

Watson, T. F., Tandem-scanning microscopy of slow speed enamel cutting interactions. *J. Dent. Res.* **70**(1): 44–49; 1991a.

Watson, T. F., Applications of confocal scanning optical microscopy to dentistry. *Br. Dent. J.* **9**: 287–291; 1991b.

Watson, T. F., Billington, R. W., and Williams, J. A., The interfacial region of the tooth/glass ionomer restoration: A confocal optical microscope study. *Am. J. Dent.* **4**(6): 303–310; 1991.

Watson, T. F. and Wilmot, D. M. deJ., A confocal microscopic evaluation of the interface between Syntac adhesive and tooth tissue. *J. Dent.* **20**: 302–310; 1992.

Watson, T. F., Petroll, W. M., Cavanagh, H. D., and Jester, J. V., *In vivo* confocal microscope in clinical dental research: an initial appraisal. *J. Dent.* **20**: 352–358; 1992.

Watson, T. F., Applications of high-speed confocal imaging techniques in operative dentistry. *Scanning* **16**: 168–173; 1994.