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Morphological Study of the Effects of CO_2 Laser Emitted at 9.3 μm on Human Dentin

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ABSTRACT

Objective*: The purpose of this study was to investigate the effects of dentin ablation using a carbon dioxide (CO_2) laser emitted at 9.3 μm by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). *Background Data*: There have been no reports on effects of CO_2 laser irradiation emitted at 9.3 μm on dentin by SEM and CLSM. *Methods:* Thirty extracted human teeth showing no clinical signs of caries were used. All teeth were horizontally sectioned to approximately 200 μm thickness and sections were irradiated using a 9.3 μm CO_2 laser at different parameters as follows: 26 mJ [energy density (ED) 53.0 J/cm^2] and 30 mJ (ED 61.1 J/cm^2). After laser irradiation, samples were treated with sodium hypochlorite, stained using rhodamine-123, and observed with CLSM followed by SEM procedures. *Results*: No craters or cracks were observed, but many small molten and rehardened particles were documented on the sample surface using SEM. Some small cracks were seen in the subsurface layer, and some patent dentinal tubules were detected using CLSM. *Conclusion*: These results suggest that laser irradiation at these parameters affected the sample surface only (less than 20 μm) and would be less harmful to thermal damage of dental pulp for dentin ablation.

INTRODUCTION

Since the development of the ruby laser by Maiman[1] and the application of this laser in dentistry by Stern and Sognnaes,[2] many researchers have studied and examined laser effects on dental hard tissues.[3,4] Effects of carbon dioxide (CO_2) laser irradiation on dental hard tissues have been investigated,[5] and many studies have been published.[4,6–8] However, continuous-wave CO_2 laser irradiation tends to char a tooth surface and overheat the pulpal chamber.[9,10] Short-pulsed CO_2 laser irradiation at appropriate parameters may be suitable for ablation of hard biological materials under appropriate conditions.[11–14]

It was reported that the inhibitory effects of low-energy pulsed-laser irradiation on the formation of artificial caries lesions in human dental enamel and dentin surfaces using the CO_2 laser at wavelengths from 9.3 through 10.6 μm were measured.[15–17] Scanning electron microscopic (SEM) studies showed that the surface effects were highly wavelength-dependent in this region[18] and that wavelengths of 9.3 and 9.6 μm were highly efficient at heating the enamel surface, much more so than even the 10.6 μm wavelength.[19] All of these studies suggest that the CO_2 laser, preferably tuned to the highly absorbed 9.3- and 9.6-μm wavelengths, may be suitable for preventive dental applications.

Recently, CO_2 lasers that deliver light in the 9.3-μm region of the infrared spectrum have also been developed for clinical use; 9.3 μm matches the absorption characteristics of hydroxyapatite well, providing good surface modification characteristics in hard tissues and consequently minimal thermal effects in adjacent tissues at appropriate parameters, and improved protection for pulpal tissues. Technological advances have now allowed manufacture of a coherent beam delivery system for this wavelength.[20,21]

The purpose of this study was to investigate the surface and subsurface effects of dentin ablation by a CO_2 laser emitting at 9.3 μm at the parameters of 26 and 30 mJ/pulse, which was the minimum limit to observe and the maximum limit in the laser device used to perform SEM and confocal laser scanning microscopy (CLSM), and then to identify possible applications for this 9.3-μm CO_2 laser to clinical treatment.

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MATERIALS AND METHODS

Sample preparation

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

Laser device and irradiation

This study was performed using a Duolase 9.3™ (Medical Optics, San Diego, CA) emitting at 9.3 μm. This laser used a hollow tube delivery system with a focusing handpiece. The parameters used were as follows: 26 mJ/pulse [energy density (ED) 53.0 J/cm^2], and 30 mJ/pulse (ED 61.1 J/cm^2); pulse duration 300 μsec; spot size 0.25 mm (diameter). These parameters were identified after irradiation at a range of parameters in preliminary investigations (unpublished observation). Every sample was clamped and exposed to one pulse only of laser irradiation at a right angle.

Staining procedure

After laser irradiation, samples for CLSM and SEM were pretreated with sodium hypochlorite (NaOCl) (5.25% by Wt, Darrow Comp., CA) for 1 hr under vacuum and ultrasonication, and then were stained using rhodamine-123 (Eastman Kodak Co., New York, NY) at a concentration of 10^{-5} *M* in phosphate-buffered saline (PBS) for 1 hr under vacuum and ultrasonication.[8,22] After staining, sections were washed two to three 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). A 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 0 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, Osaka, Japan) and three-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 the observation using CLSM, the samples were dehydrated in a graded series of aqueous ethanol (30, 50, 70, 90, and 100% ethanol) for 30 min at each concentration, mounted on stubs using colloidal silver liquid (Ted Pella, CA), and gold coated on a PAC-1 Pelco advanced coater 9500 (Ted Pella, CA). Micrographs of the dentin surface were taken on a Philips 515 (Mohawk, NJ) SEM.

RESULTS

Figure 1A and B, shows representative surface effects seen by SEM after irradiation at 26 mJ. The residual surfaces showed ablation and melting, but no crater and cracks were evident. There appeared to be many small molten and rehardened particles in the irradiated area. A few dentinal tubules and the holes like stream bubbles appeared patent in the previously molten particles, whereas most dentinal tubules appeared patent, except in the underlying dentin surface (Fig. 1b). Additionally, a white marginal frosted zone was evident around the periphery of the irradiated area (Fig. 1a). Figure 2a–d, depict representative CLSM photographs of samples irradiated at the same parameter. The surfaces of irradiated areas again appeared ablated and melted, and many dentinal tubules in these zones were patent (Fig. 2b, c), with a similar appearance as in Fig. 1. Additionally, a white marginal frosted zone was evident around the periphery of the irradiated area (Fig. 2a and b). Some small

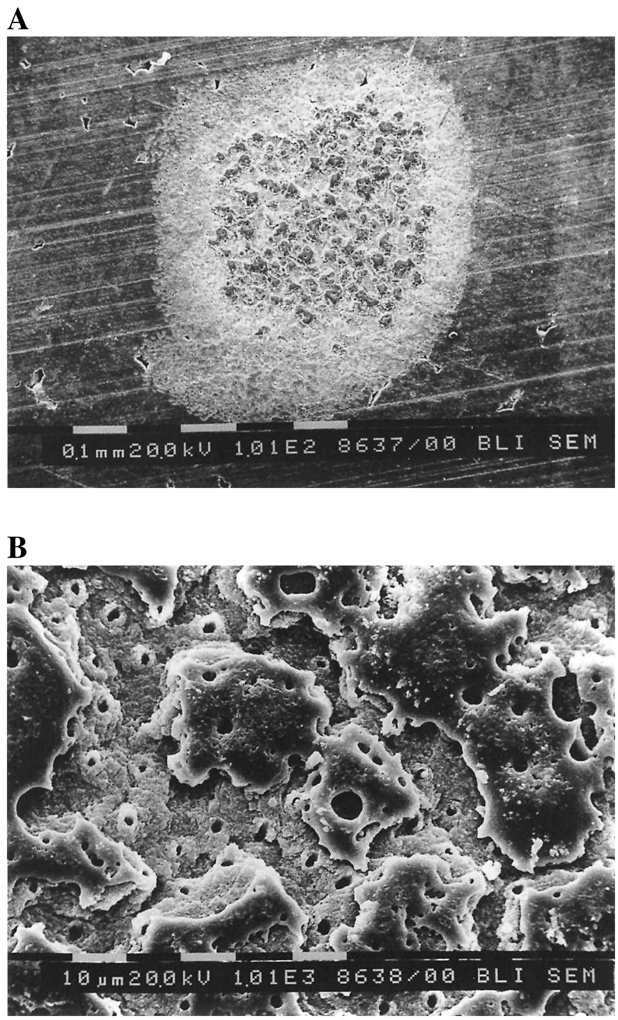

FIG. 1. Representative SEM photographs of samples irradiated at 26 mJ. (**A**) Magnification of ×101. (**B**) Magnification of ×1,010. The bar represents 0.1 mm (**A**), or 10 μm (**B**).

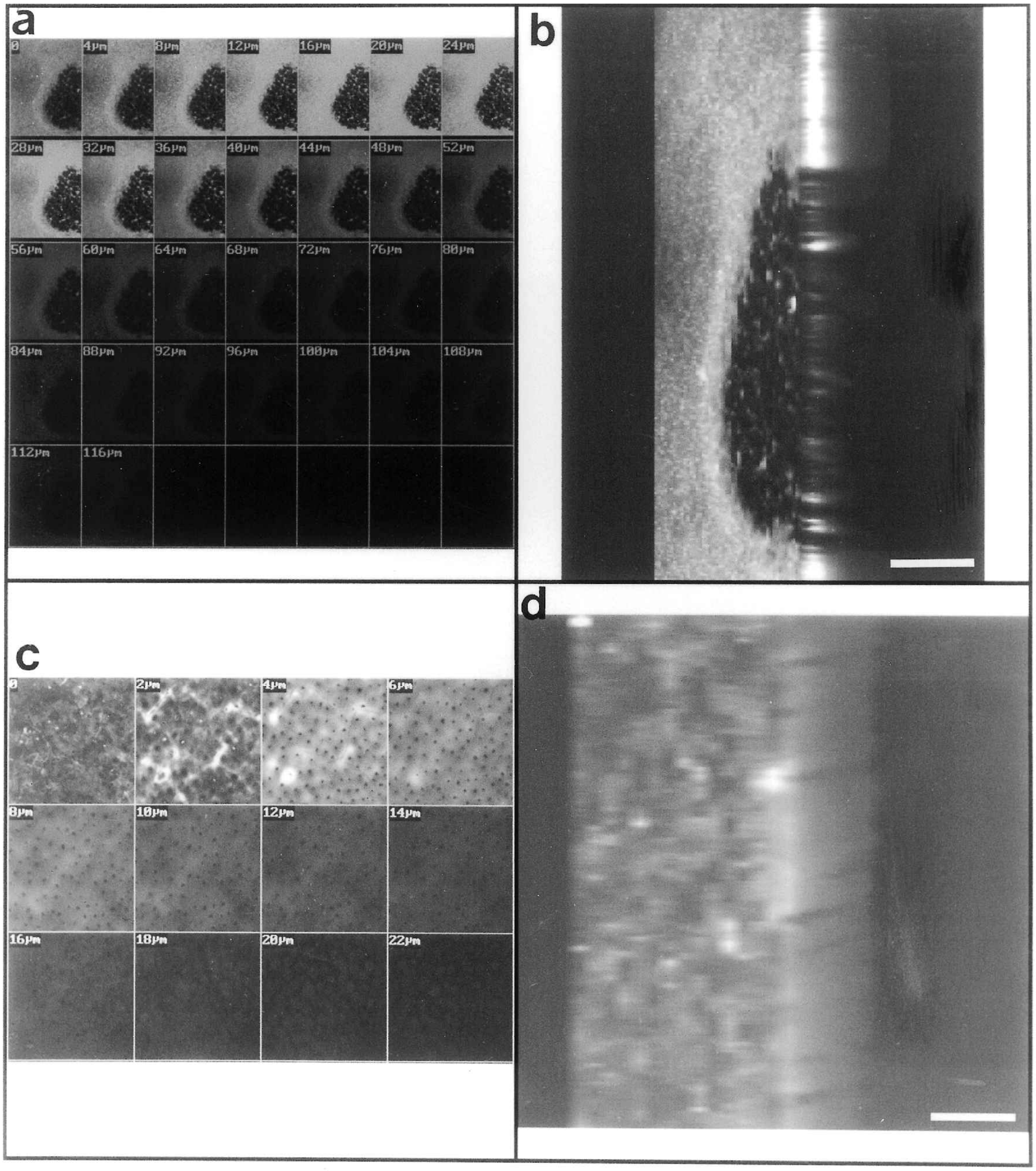

FIG. 2. Representative CLSM images of samples irradiated at 26 mJ. (**a, b**) Magnification of ×100. (**c, d**) At a magnification of ×1,000. (**a**) Series of horizontal sections made at 4-μm intervals. (**b**) The three-dimensional lateral view (angle 70°). (**c**) Series of horizontal sections made at 2-μm intervals. (**d**) The three-dimensional lateral view (angle 70°). The bar represents 100 μm (**b**) or 10 μm (**d**).

cracks in the 2- to 4-μm-deep subsurface layer of the irradiated area were evident at high magnification (Fig. 2c). Figure 2d is relatively poor due to the inherent resolution limitations of the method used. Figure 3 a and b, show representative SEM images in samples irradiated at 30 mJ. The altered area was larger than after irradiation at 26 mJ, the marginal frosted zone was somewhat narrower (Fig. 3A). On the surface, ablation and melting were apparent and neither small crack-like lesions nor mini-craters were observed (Fig. 3A, B). There were many molten and rehardened particles in the irradiated area (Fig. 3A, B). A few dentinal tubules and holes like stream bubbles appeared patent in these molten and rehardened particles more than in the samples irradiated at 26 mJ, and most dentinal tubules appeared patent in the underlying dentin surface (Fig. 3B). Figure 4a–d, shows representative CLSM images of samples irradiated at the same parameter as in Fig. 3. On the surface, some dentinal tubules in the irradiated area appeared open (Fig. 4b, c) as in Fig. 3, but, at a subsurface level below 16 μm, most dentinal tubules

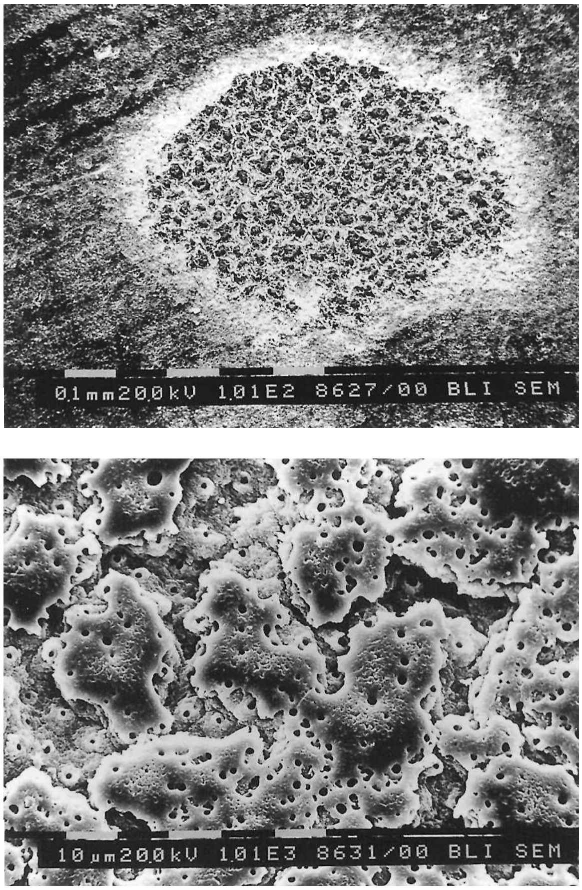

FIG. 3. Representative SEM photographs of samples irradiated at 30 mJ. (**a**) Magnification of ×101. (**b**) Magnification of ×1,010. The bar represents 0.1 mm (**a**), or 10 μm (**b**).

appeared closed (Fig. 4c), and dye penetration in these areas was poor (Fig. 4a–d). Some small cracks at 2–6 μm in the subsurface layer of the irradiated area were evident at high magnification (Fig. 4c). A few dentinal tubules in this zone were still patent, but dye penetration into this zone was less than that into the nonirradiated area (Fig. 4b). Figure 4d was relatively poor due to the inherent resolution limitations of the method used, because dye penetration was poor.

DISCUSSION

In this study, the surface and subsurface effects of laser-induced ablation of dentin were investigated using SEM and CLSM morphologically *in vitro*. Neither cratering nor cracking were apparent on the sample surfaces, and some molten and resolidified dentin particles were detected in the irradiated area using SEM. However, small cracks were identified in the subsurface layer using CLSM techniques. Laser-induced effects at 30 mJ exceeded those documented at 26 mJ: more ablation and melting and closed or narrower dentinal tubules at a subsurface level were seen in samples irradiated at 30 mJ than in those treated at 26 mJ. Though thin caps of fused material covering the tops of the tag-like projections within dentinal tubules[16] were not seen in this study, closed or narrowed tubules were observed to an increasing extent at greater depths within the dentin. Most of the irradiated energy in this study seemed to be concentrated at the surface (less than ~20 μm) judging from CLSM photographs compared to that by a 10.6-μm CO_2 laser.[8] Similar results were reported in enamel.[18,23] An absorption depth of a 9.3-μm CO_2 laser was less than that of a 10.6-μm CO_2 laser, and reflectance by a 9.3-μm CO_2 laser was higher than that of a 10.6-μm CO_2 laser. These results suggest that a 9.3-μm CO_2 laser may have advantageous (decreased) thermal effects.[14]

The thermal effect was not examined in this study, but considering other reports,[14,19,24] the thermal effect of this 9.3-μm CO_2 laser on pulp was thought to be less than that of the conventional CO_2 laser. From measurements of time-resolved surface temperature of laser-irradiated bovine enamel at 9.3, 9.6, 10.3, and 10.6 μm, the temperature was highest at 9.3 μm followed by a 9.6 μm,[14] but considering the physical properties (reflectance, absorption coefficient, and absorption depth) of this laser on enamel and dentin, the thermal effect on pulp also might be less.[18,23]

The detection of ablated dentin by laser was achieved using CLSM in this study. CLSM might be useful for laser-induced ablation study, because CLSM allows surface and subsurface visualization in three dimensions using a fluorescent dye.[8,22,25,26] Compared with SEM, the magnification provided by confocal microscopy is low (maximum ×1,000). 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.[27] 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, because relatively thin sample sections are needed. However, the high-frame speed of the tandem scanning microscopy (TSM) enables real-time examination of teeth *in vivo*.[28,29]

In another experiment of atomic analyses, calcium (Ca) and phosphorus (P) contents on the dentin slice surfaces were increased significantly after low-level laser irradiation, but a ratio of Ca to P was almost the same level as the control (nonirradiated area) ($p > 0.01$).[30] This result suggests that not only recrystallization but also an increase of inorganic content occur in the laser-irradiated dentin surface. This might be related to less demineralization.[16,31]

Sliced samples were used in this study, because sliced samples are suitable for CLSM observation.[8,22,25,26] Dye is easy to penetrate into samples, and flatter surfaces are better for CLSM observation. If laser irradiation is performed on a flat surface at a right angle, irradiated laser energy is easy to absorb or penetrate into samples. Dramatic changes at sliced samples could be observed at a relatively low laser energy.

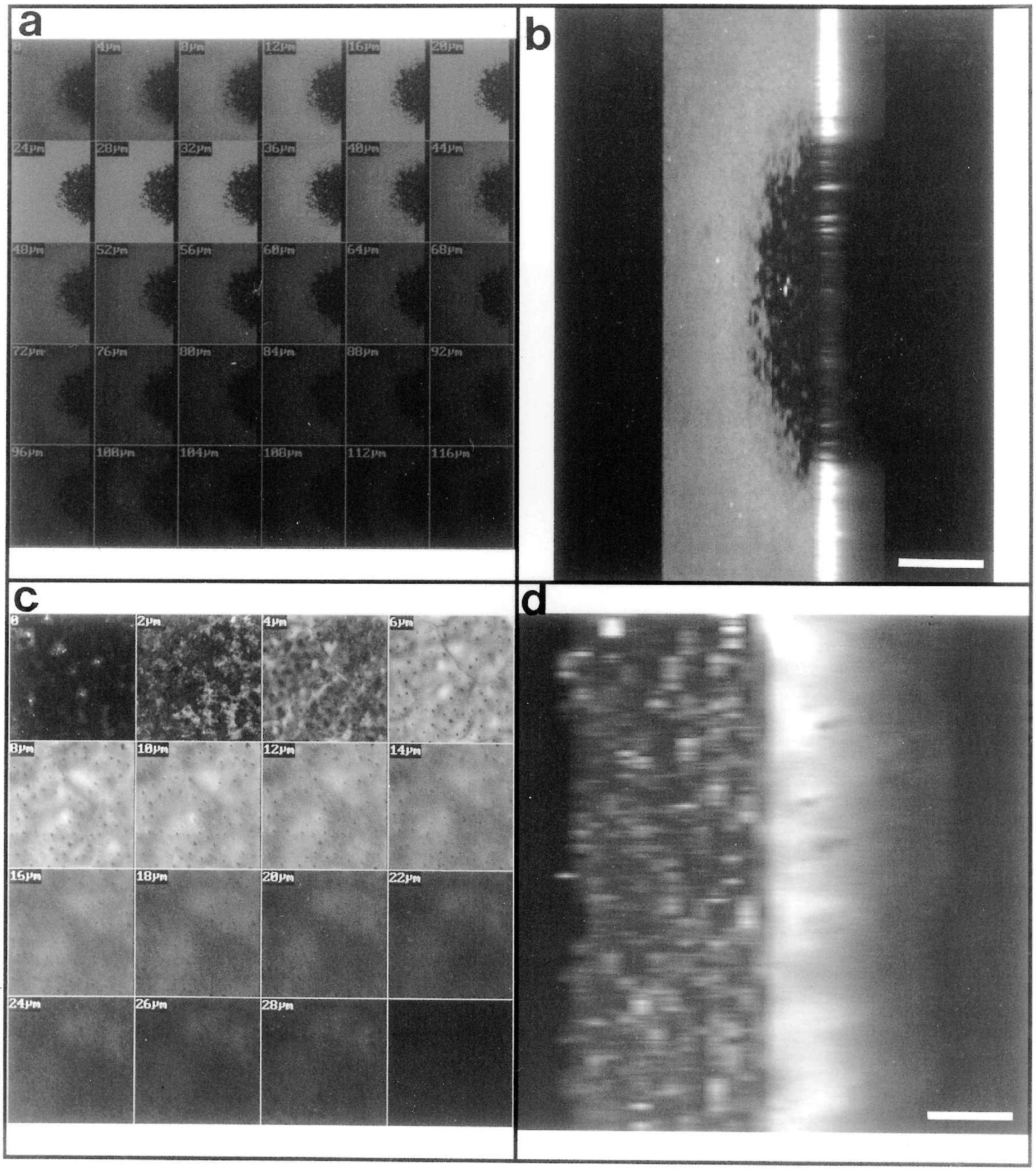

FIG. 4. Representative CLSM images of samples irradiated at 30 mJ. (**a,b**) Magnification of $\times$100. (**c,d**) Magnification of $\times$1,000. (**a**) Series of horizontal sections made at 4-μm intervals. (**b**) The three-dimensional lateral view (angle 70°). (**c**) Series of horizontal sections made at 2-μm intervals. (**d**) The three-dimensional lateral view (angle 70°). The bar represents 100 μm (**b**) or 10 μm (**d**).

Further ablation study at different parameters and study for thermal damage will be needed prior to clinical application.

CONCLUSION

These results suggest that a 9.3-μm CO_2 laser is less harmful to thermal damage of dental pulp for dentin ablation, because laser irradiation at these parameters affected the sample surface only (less than 20 μm). 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.

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