Measurement of crater geometries after laser ablation of bone tissue with optical coherence tomography

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The feasibility of measuring crater geometries by use of optical coherence tomography (OCT) is examined. Bovine shank bone on a motorized translation stage with a motion velocity of 3 mm/s is ablated with a pulsed CO_2 laser *in vitro*. The laser pulse repetition rate is 60 Hz and the spot size on the tissue surface is 0.5 mm. Crater geometries are evaluated immediately by both OCT and histology methods after laser irradiation. The results reveal that OCT is capable of measuring crater geometries rapidly and noninvasively as compared to histology. There are good correlation and agreement between crater depth estimates obtained by two techniques, whereas there exists distinct difference between crater width estimates when the carbonization at the sides of craters is not removed.

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Lasers have found access to a variety of medical fields over the years. One of the fields of interest is to incise and excise hard tissue by use of laser ablation effects. In clinical applications, one of the most important problems is to maximize ablation rates and minimize peripheral thermal damage. At present, the main way to evaluate ablation rate and thermal damage is histology, for which the tissue sample needs a series of processes, such as fixation, dehydration, and decalcification. This sort of processes is cumbersome and destructive. Also, it must be performed by the professional. Therefore, a new method is strongly required, which can identify the crater morphology and thermal damage more conveniently and accurately.

Optical coherence tomography (OCT) is an emerging noninvasive, noncontact, and near real-time imaging modality[1]. OCT devices measure the intensity of backscattered light at different positions inside the specimen. At present, it is widely used in scientific research and clinical medicine[2−5]. Torkian *et al.* compared OCT with conventional histology in evaluation of the crater dimensions in fresh porcine vocal cords[3]. The experiment results illustrated the ability of OCT to accurately depict key features of native and damaged vocal fold mucosa and showed good correlation and agreement between estimates obtained by two methods. However, the studies of comparison between two methods on hard tissue have not yet appeared. In this letter, the crater geometries of fresh bone tissue obtained by both OCT and histology methods are compared. Our main goal is to determine the ability of OCT to evaluate destructive lesions created by the surgical carbon dioxide laser in bone tissue using histology as a standard.

Bovine shank bone was used as the specimen material. It was obtained from a local slaughter house no later than six hours postmortem, then excised to pieces (about 40×20×5 (mm)). Areas of the bone surface exhibiting a clean and smooth cortical surface were prepared by scraping the surface with a razor blade to remove the periosteum. Then the tissue pieces were immediately wrapped in a normal saline-soaked gauze and kept in a refrigerator at around 4° before laser irradiation. Prior to laser irradiation, the tissue pieces were allowed to reach room temperature.

The laser source used here was a pulsed CO_2 laser (Sharplan, 30C, Israel, $\lambda = 10.64\ \mu$m). Its repetition rate was 60 Hz and its power ranged from 1 to 30 W. The exact radiant exposure was determined by reading the laser pulse energy with a pyroelectric detector and relating to the beam area. The specimen was put on the motorized translation stage before irradiation and the position of tissue surface was adjusted. As the CO_2 laser was emitted, the samples were moved perpendicularly through the laser beam so as to obtain a linear incision on the bone tissue. The experimental setup is shown in Fig. 1. The geometrical pulse overlap factor n of the laser pulses on the tissue is calculated by

$$n = fw/v, \tag{1}$$

where f is the pulse repetition rate, w is the beam radius, v is the velocity of motorized translation stage. The beam diameter on the tissue surface is 0.5 mm and the

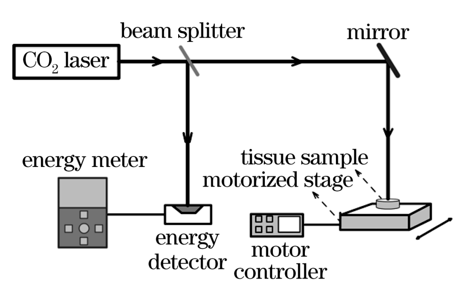

Fig. 1. Schematic of the experiment setup.

velocity of motorized translation stage is 3 mm/s, which means a corresponding pulse overlap of 5.

After irradiation, the carbonization in the bottom of ablation craters was removed by the ethanol-soaked cotton, while the carbonization at the sides of craters remained. Laser-induced craters were examined by OCT system with the lateral and axial resolution of ~ 10 μm to obtain quantitative ablation crater dimensions. The central wavelength of the light source used in the OCT system is 842.5 nm, and the bandwidth is 49.2 nm. The output power is 7 mW. After OCT imaging was completed, the tissue was fixed in 10% formaldehyde for 24 h, decalcified in HNO_3 solution, dehydrated in progressive concentrations of ethanol, embedded in paraffin, cut into 4-μm-thick serial sections, and finally stained with hematoxylin and eosin. Then the serial tissue sections were examined by confocal microscopy (Zeiss, LSM 510 META, Germany) to measure the crater geometries.

The relationship between OCT and histology estimates of the crater dimensions was determined by linear regression and Pearson's correlation. Bland-Altman analysis was used to determine the agreement between two estimates[6].

OCT images of bone tissue obtained immediately after laser ablation are compared with the corresponding histologic sections in Fig. 2. The location and overall extent of tissue destruction are readily apparent, where the deepest is formed at the center of ablated craters.

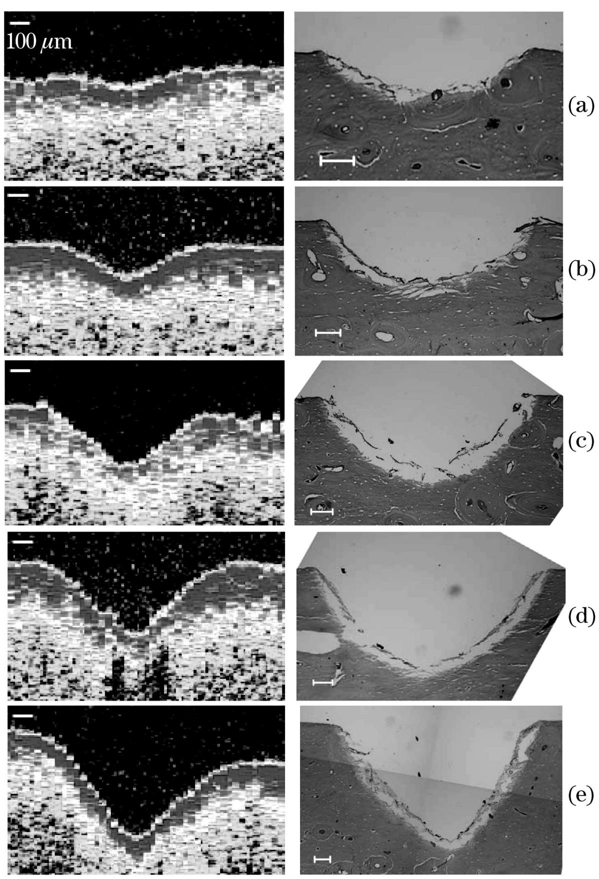

Fig. 2. Bovine shank bone after laser ablation at (a) 5.7, (b) 15.4, (c) 33.3, (d) 41.2, and (d) 44.6 J/cm^2. The OCT images are shown in the left, and the corresponding histologic images in the right. The length of bar is 100 μm.

The different gray values in OCT images are thought to be due to the differences in refractive indices as light passes from air to the tissue.

Crater depth and width determined with both OCT and histology versus laser fluence are reproduced in Fig. 3. For the two methods, estimates of crater depth and width are directly proportional to laser fluence, which is in accordance with previous studies[2,7,8]. Crater depth estimates by two methods are almost equal in Fig. 3(a), except at 44.6 J/cm^2 where the estimate by histology is evidently bigger than that by OCT. In Fig. 3(b), the estimates of crater width by histology are markedly bigger than those by OCT. Moreover, the diversity

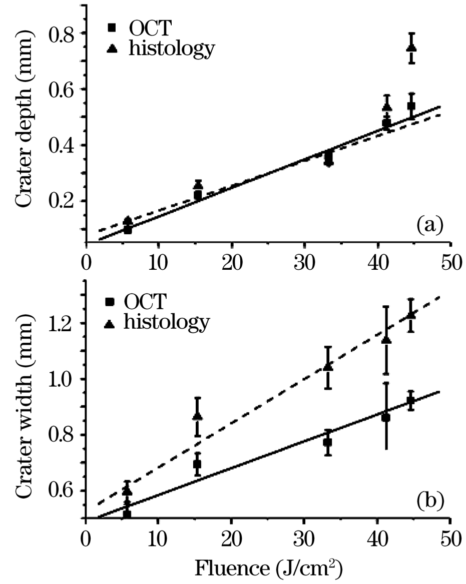

Fig. 3. (a) Crater depth and (b) crater width as functions of fluence as measured by OCT or histology. The error bars are standard deviations of data.

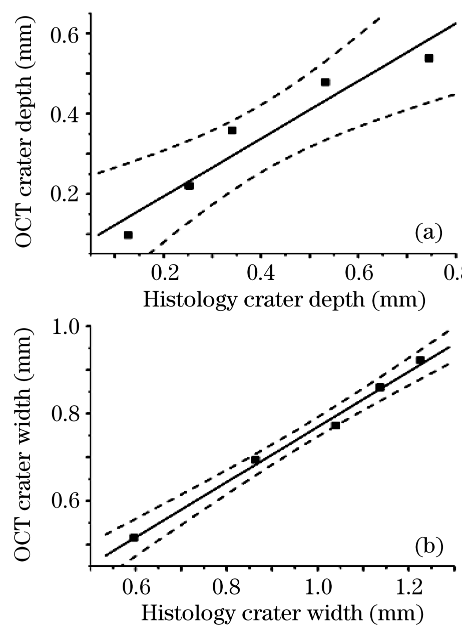

Fig. 4. OCT and histology estimates of (a) crater depth and (b) width as functions of laser fluence. Solid line is the linear regression line, and dashed lines indicate 95% confidence intervals.

between two measurements increases with laser fluence.

As shown in Fig. 4, there is good correlation between estimates by two techniques for both crater depth ($R = 0.96$, $P = 0.009$) and width ($R = 0.99$, $P < 0.001$).

The agreement between OCT and histology for determining crater depths and widths are presented in Fig. 5. The results from Bland-Altman analysis are summarized in Table 1. For both crater depth and width, estimates by histology are bigger than those by OCT, except at 33.3 J/cm^2 where the estimate of crater depth by histology is less than that by OCT. The average difference between estimates of crater depth by two methods is 0.06 mm and that for crater width is 0.22 mm.

In this letter, a direct comparison of OCT to conventional histologic imaging of laser-ablated shank bone illustrates the ability of OCT to accurately measure crater geometries. OCT has the following advantages as compared to histology. Firstly, it does not make any changes on tissue; secondly, the process of measurement is easy to perform. However, the tissue structure corresponding with the layer on OCT image cannot be interpreted clearly. At present, OCT devices have been used to study the structure of different tissues, such as skin, vocal cords, dentin etc., and identify the natural and pathological tissues[3−5].

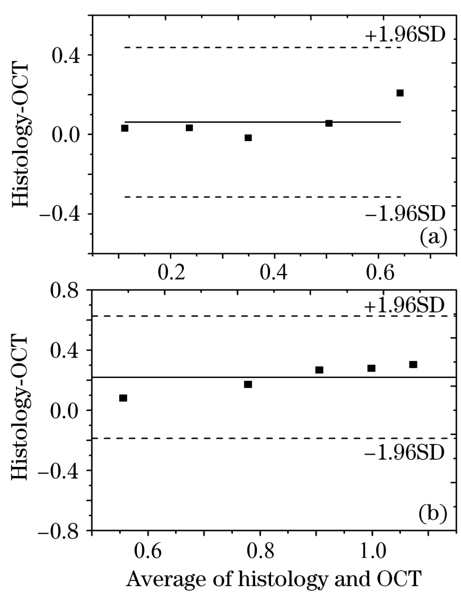

Fig. 5. Agreement between OCT and histology estimates of (a) depth and (b) width by Bland-Altman analysis. Solid line indicates mean difference between histology and OCT, and dashed lines indicate the limits of agreement (mean difference, ±1.96 standard deviation (SD)).

Table 1. Bland-Altman Analysis of Morphologic Characteristics of the Ablation Craters, Histology versus OCT

	Limits of Agreement	
	Lower Limit (−1.96SD; mm)	Upper Limit (+1.96SD; mm)
Depth	−0.31	0.44
Width	−0.19	0.62

The acquisition of data required capturing the crater depth at the deepest position of ablation crater in either a histologic section or an OCT image. Moreover, the data must be obtained at the same position of ablation crater by two methods. In order to solve the above difficulties, the bone tissue was put on a motorized translation stage in our study. By choosing the proper velocity, the ablation crater was obtained, whose depth and width were the same at different positions of a linear cut[9,10]. So the influence of position was reduced. Furthermore, in Figs. 3 and 5, there is obvious difference between two estimates of crater width. It may be due to the removal of ablation debris and carbonization in the process of histologic section. Moreover, histologic sectioning of the sample may distort the tissue section and result in additional measurement errors.

In conclusion, OCT is capable of measuring crater geometries rapidly and noninvasively as compared to histology. There are good correlation and agreement between estimates of crater depth by two techniques.

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