Histological Observation on Dental Hard Tissue Irradiated by Ultra-short Pulsed Laser

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超短パルスレーザー照射による硬組織の組織学的評価

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要旨: 近年, レーザーパルス幅がピコ秒からフェムト秒オーダーである超短パルスレーザーが様々な分野に応用されている。このレーザーは, 最小限の熱影響で, 金属や半導体, 高分子材料そして生体組織を加工することが可能である。歯科分野において, 既存のレーザーシステムでの治療もしくは基礎研究から, 硬組織の熱影響またはクラック発生などの副作用が報告されている。記述した特性を持っている超短パルスレーザーは, このような問題の解決を期待できるため, 硬組織への超短パルスレーザー照射効果の有効性が研究されている。本研究では, 歯牙硬組織への超短パルスレーザーの照射効果の有効性及び安全性を評価するために, 超短パルスレーザー照射前・後の歯牙硬組織の形態的な変化と光学的な変化を調べた。照射対象はプレート状に加工したウシ歯エナメル質・象牙質であった。レーザーは, 発振波長 800nm, 繰り返し周波数 1 kHz, パルス幅 130fsec の Ti: sapphire レーザーを使用した。レーザーの平均パワー密度は, 90, 180, 360W/cm² であった。レーザー照射後の対象の照射表面は SEM と EDX を用いて評価した。対象の光学的変化の評価は, FTIR を用いた対象の吸収スペクトル変化の観察によって行った。SEM 観察によって, 超短パルスレーザーの照射は, 硬組織表面の炭化及びクラックを誘起せずに切削が可能であることが明らかとなった。EDX 計測において, レーザー照射前・後の硬組織の P/Ca は変化しておらず, また, FTIR 計測では赤外吸収スペクトルの変化は照射前・後で変化は見られなかった。レーザー照射の場合, これらの変化が起こる要因は, 主に熱的な影響であることが報告されていることから, 超短パルスレーザーは熱影響を最小限にして硬組織を切削できることが明らかとなった。

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Key words = Ultra-short pulsed laser, Ablation, Thermal damage

キーワード = 超短パルスレーザー, アブレーション, 熱影響

Introduction

Recently, ultra-short pulsed laser (USPL) has been extensively studied for ablating diverse materials such as metals¹⁻³⁾, high-polymer materials⁴⁻⁶⁾, and biological tissue⁷⁻⁸⁾ because USPL can ablate materials with minimal thermal damage. In the field of dentistry, lasers are used to remove carious dental hard tissue and for cavity preparation of dental hard tissue because they offer several advantages such as no pain, no noise and low-invasiveness, over the dental high-speed drill. However, it has been reported that irradiation with the conventional laser system induces thermal damage such as carbonization and micro-cracks⁹⁻¹²⁾ in dental hard tissue. These side effects occur because the laser pulse duration is longer than the thermal relaxation time of tissue. In other words, a laser pulse, which is of shorter duration than the thermal relaxation time of tissue, can ablate tissue without thermal side effects. Therefore, USPL, which has the laser pulse duration of femto¹⁰⁻¹⁵⁾ to pico¹⁰⁻¹²⁾ seconds, is expected to remove carious dental hard tissue and cavity preparations of dental hard tissue without thermal side effects. In fact, Niemz has demonstrated the effectiveness of USPL irradiation in dentistry in principle¹³⁾. He clarified that USPL irradiation caused no thermal damage to dental hard tissue using Nd:YLF picosecond laser. In addition, Weigl et al. studied the application of USPL to dentistry with Ti:sapphire laser of femto second pulse duration¹⁴⁾. However, they have not investigated the optical changes of samples irradiated with USPL, although such measurements are commonly made in basic research of laser dentistry. In this paper, we examine the optical change of samples irradiated with USPL and observe dental hard tissue irradiated with USPL.

Materials and Methods

Ti:sapphire femto second laser

Ti:sapphire laser (haricain, Spectra-Physics, Inc.) was used in this study. The laser had a wavelength of 800 nm, pulse duration of 130 femtosecond, pulse repetition rate of 1 kHz, and average power density of 90, 180, 360W/cm². These average power densities were measured using power meter (Model 407A, Spectra-Physics, Inc.). Laser spot size was 200μm × 200μm, which was calculated by theoretical analysis of lens focus number.

Figure 1 Separation method of enamel and dentin. Separation of dental enamel and dentin was achieved using diamond cutter

Tooth preparation

The tooth used for this study was enamel plates and root dentin plates of extracted bovine teeth. Figure 1 shows the separation method of enamel and root dentin. These plates were processed to the size of 1 × 2 × 0.5 (cm) using a cutter. The surfaces of plates were polished using sandpapers of #400 to #1200. N numbers of samples were each subjected to laser irradiation three times. These plates were irradiated with USPL using a stage moving at 1 mm/sec.

Morphological and histological observation of irradiated dental hard tissue

A scanning electron microscope (SEM) was used to examine the morphological change of irradiated sample. The observation part were the surface and the cross surface of irradiated samples. SEM images of samples were observed at an applied voltage of 20kV after the samples were evaporated with platinum.

Energy dispersive X-ray analysis (EDX) was used for examining the histological change of irradiated sample. EDX can determine quantitatively the structural composition of objects. In this study, the histological changes of irradiated samples were determined by comparing the phosphorus (P)/calcium (Ca) ratio of irradiated samples with those of non-irradiated samples.

Measurement of optical change

Fourier Transform Infrared Spectroscopy (FTIR) was used to observe the optical change of samples irradiated with USPL. FTIR can measure the optical absorption of objects, spectrally. The measurement conditions of FTIR were a resolution of 2 cm⁻¹ and gain of 4. The

Figure 2 SEM image of the dentin samples irradiated with ultra-short pulsed laser. In the upper image, magnification is $\times 500$ and bar is $10\mu\text{m}$. In the lower image, magnification is $\times 3000$ and bar is $1\mu\text{m}$

optical change of irradiated sample was determined by comparing the absorption spectrum of irradiated samples with non-irradiated samples.

Result

Figure 2 shows the surface of a dentin sample irradiated with USPL. The dentin was sharply and straightly ablated by scanned laser irradiation, not roughly like Er:YAG laser. In addition, dentinal tubules were opened clearly. There was no smear layer or carbonized layer on the surface of irradiated dentin.

Figure 3 shows the cross section of a dentin sample ir-

Figure 3 SEM image of the cross section of dentin samples irradiated with ultra-short pulsed laser. In all images, the magnification is the $\times 500$. Average power densities are $90\text{W}/\text{cm}^2$ (upper), $180\text{W}/\text{cm}^2$ (middle), and $360\text{W}/\text{cm}^2$ (lower)

Figure 4 SEM image of the surfaces and cross section of enamel samples irradiated with ultra-short pulsed laser. Each image has a magnification of $\times 500$ (a), $\times 2500$ (b), $\times 5000$ (c), and $\times 1000$ (d). Images (a), (b), and (c) show the surface enamel irradiated with USPL. Image (d) is a cross section

radiated with USPL at 90, 180, 360W/cm². The ablation depth by USPL irradiation increased in proportion to the laser power density. Dentin fragments and micro-cracks were not observed on the irradiated samples.

Figure 4 shows the surface and cross section of an enamel sample irradiated with USPL. Like the dentin sample, the enamel samples have sharp ablation, without smear layer, carbonized layer, or crack.

The change of mass percent of irradiated samples is negligible as shown in Table 1. There was no significant difference between the irradiated sample and the non-irradiated sample, both for the dentin sample and enamel sample, by the t-test ($\alpha = 0.05$).

Table 1 Mass percent of P/Ca by EDX measurement. "Irradiated dentin or enamel" means the samples irradiated with femto second laser. "Control dentin or enamel" means non-irradiated samples

Mass percent of P/Ca	
Samples	Ratio of P/Ca
Control dentin	0.488 $\pm$ 0.01
Irradiated dentin	0.484 $\pm$ 0.013
Control enamel	0.468 $\pm$ 0.006
Irradiated enamel	0.462 $\pm$ 0.008

Error is standard deviation of 10 measurements

Figure 5 IR spectrum of the irradiated sample. The upper spectrum is for the dentin irradiated with USPL and non-irradiated dentin. The lower spectrum is the case of enamel samples

Figure 5 shows the infrared absorption spectrum of irradiated samples and non-irradiated samples. There was no change in the absorption peak of PO_4^{2-} , which is a steep peak in the $9\mu\text{m}$ region, between the irradiated sample and non-irradiated sample, both for the dentin and enamel. In addition, the peak of $6\mu\text{m}$ region had no change and no shifting, too. These peaks are amideI, amideII.

Discussion

Laser ablation of dental hard tissue can be achieved using Er:YAG laser and the $9\mu\text{m}$ region CO_2 laser that is absorbed in PO_4^{2-} of hydroxyapatite¹⁵⁾. However, it has been reported that laser pulse duration, which is longer than the thermal relaxation time of dental hard tissue, lead to thermal side effect to teeth. These thermal

side effects can be observed by morphological, histological, and optical measurement, etc. In morphological measurement such as SEM, thermal side effects can be observed as a carbonized layer and thermal cracks. Histological observation such as EDX can confirm thermal effects by observing the change of P/Ca ratio in dental hard tissue. Optical measurement such as FTIR can observe thermal side effects by comparing the spectrum of an irradiated sample with that of a non-irradiated sample. Niemz and Weigl has shown the ablation method that had the no thermal side effect to ablated dental hard tissue using ultra short pulsed laser such as pico, femtosecond laser^{13,14)}. They used morphological measurement mainly. In this study, we investigated ultra-short pulsed laser-dental hard tissue interaction using FTIR as optical measurement, EDX as histological measurement, and SEM. These measurements showed no optical change, no crack, no carbonized layer, no change of P/Ca ratio, and opened dentinal tubules. From these results we confirmed that the ultra-short pulsed laser can ablate dental hard tissue with no crack, no fragment, and no thermal damage. These results confirm the reports^{13,14)} and are expected to resolve the thermal side effects to hard tissue caused by the conventional laser.

Tooth dentin consists of 70% hydroxyapatite, 12% water, and 18% organic matter. On the denaturation of organic matter, it has been reported that ultra-short pulsed laser could ablated the brain sample as organic matter with no thermal side effect⁷⁾. In addition, we had confirmation of the no change of absorption peak of $6\mu\text{m}$ region in the dentin samples. These evidences confirm that ultra-short pulsed laser has not the thermal side effect on soft tissue.

It has been reported that the solution layer of dental hard tissue surface irradiated with conventional laser such as CO_2 can increase the acid resistance of dental hard tissue¹⁶⁾. However, USPL is not expected increase of acid resistance because the sample surface irradiated with USPL had no solution layer.

Conclusion

The effectiveness of ablation on dental hard tissue using an ultra-short pulsed laser (USPL) was investigated by morphological, histological, and optical observations. The results clarified that:

1) There was no optical change of dental hard tissue after USPL irradiation.

- 2) The dental hard tissue was ablated, sharply.
- 3) Dentinal tubules were clearly opened at the surface of irradiated dentin.
- 4) Carbonization and cracks were not observed in the irradiated samples. Therefore, USPL is expected to use as an ablation method without causing thermal damage to dental hard tissue. Future studies will be needed to clarify the relationship between laser energy and ablation ability with USPL, and the effectiveness of ablation for carious teeth.

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