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Novel Laser Therapy and Diagnosis using Mid-infrared Laser

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Abstract— **Mid-infrared (MIR) laser with a specific wavelength can excite the corresponding biomolecular site to regulate chemical, thermal and mechanical interactions to biological molecules and tissues. In laser surgery and medicine, tunable MIR laser irradiation can realize the safety regulation of therapeutic effect, less-invasive treatments and the special diagnosis by vibrational spectroscopic information. This paper showed a novel therapeutic and diagnostic applications using tunable MIR laser.**

I. Introduction

IN the last decade, many infrared (IR) lasers with several wavelengths were developed based on the enlargement of oscillating wavelengths, particularly long wavelength. These correspond to Nd:YAG laser (1.064 µm), Ho:YAG laser (2.1 µm), Er:Cr:YSGG laser (2.79 µm), Er:YAG laser (2.94 µm) and CO_2 laser (10.6 µm etc.) and so on. These lasers can induce a chemical effect, a thermal effect and ablations of hard and soft tissues. Examples of medical uses of these lasers are dermatology, dentistry, angiostomy, ophthalmology, otolaryngology, gastroenterology and urology and so on.

In several years, many mid-infrared (MIR) light sources wavelength tunable have appeared. Although free electron laser (FEL) is famous for the special laser system with wide range wavelength tenability, An FEL can emit a high-power laser pulse over a wide range of wavelengths. Radiation from an IR FEL with a specific wavelength excites the corresponding biomolecular site to enable the regulation of chemical, thermal and mechanical interactions [1]. The specific IR sensitivity of biomolecules such as proteins [2], sugar chains, and lipids can be exploited. Many medical applications of the FEL, angioplasty [3] for cardiovascular surgery and neurosurgery, preventive dentistry [4], ophthalmology, orthopedic surgery and so on, have been proposed. However, the researches using FEL have been limited to seed researches because it is a controlled area for radiation and a huge facility.

Recently, the developments of table-top light sources with wavelength tunability have made progress and optical parametric oscillation (OPO), difference-frequency generation (DFG) and quantum cascade laser (QCL) etc. have started to become widely used at a laboratory level. Consequently, application studies in a MIR region have gained recognitions.

MIR wavelengths correspond to the molecular stretching and vending vibrations. Thus MIR tunability means to be able to provide MIR photon energies with a specific target, molecular bonds and functional groups, by the irradiation with a wavelength corresponding to a molecular vibration. MIR laser irradiation can realize a limitation of therapeutic effects and an inhibition of side effects and leads to less-invasive treatments. In order to get the less-invasive treatment effects, it is essential to determine the molecular vibrations (i.e. IR absorption) of a target before treatments using MIR lasers.

In this paper, we introduced a novel therapeutic and diagnostic applications using tunable MIR pulsed laser by DFG method. Specifically, we showed the studies about less-invasive laser angioplasty with a wavelength of 5.75 µm and IR spectroscopy of biological samples using tunable IR laser with a wavelength range from 5.5 µm to 10 µm.

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II. Development of Less-invasive Laser Angioplasty for Atherosclerosis using Nanosecond Pulsed Laser

A. Background

Cholesteryl ester, cholesterol bound to a fatty acid such as oleic acid linked by an ester bond, is the main component of atherosclerosis. Cholesteryl ester accumulates in the arterial wall in a complicated manner. MIR laser at 5.75 µm is selectively well absorbed in C=O stretching vibration mode of ester bonds. Effective removal of cholesterol esters can be achieved by selecting the wavelength which satisfies the absorption conditions for the cholesterol ester but will not adversely affect the normal tissue. Cholesteryl esters have the characteristic absorption peak at 1739 cm^{-1} which corresponds to the C=O stretching vibration mode of ester bonds.

We had investigated the irradiation effects on cholesteryl oleate using FEL, which is a microsecond pulsed laser having wavelength tunability in MIR region. In our previous studies, we have obtained that the ester bond of a cholesteryl oleate are dissociated into cholesterol and carboxylic acid by thermal effect and depends on the amount of laser energy absorbed rather than on the excited mode. On the other hand, selective decomposition of the atherosclerotic plaque from an atherosclerotic legion has been particularly difficult with an endothelial cell layer non-invasive. Thus, the characteristic of selective ablation removal of atherosclerotic plaques with tunica intima is also important for laser angioplasty.

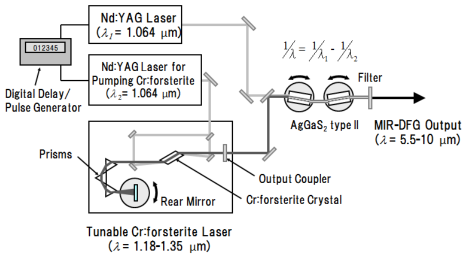

Fig. 1. Optical setup of tunable MIR laser by DFG method.

Nanosecond pulsed laser has been believed to be better suited for inducing the mechanical interaction with the wavelength selectivity.

The purpose of this study is to determine the effectiveness of nanosecond pulsed laser at 5.75 μm irradiation to atherosclerotic tunica intima in a wet condition. This study shows that nanosecond pulsed laser irradiations at 5.75 μm provide an alternative laser light source as a selective and less-invasive treatment tool for removal of atherosclerotic plaque.

B. *Experimental Setup*

1) Tunable MIR laser: The prototypes of the tunable MIR laser and the tunable Cr:forsterite laser as a part of the MIR laser was developed by RIKEN and Kawasaki Heavy Industries, Ltd. (KHI). Then, a full automation of the wavelength tuning and the stabilization of the output energy have been performed by KHI [5,6].

The tunable MIR laser in a wavelength range of 5.5–10 μm was obtained by DFG between a Q-switched Nd:YAG laser with a wavelength of 1064 nm (Tempest 10, New Wave Research, Inc., USA) and the Cr:forsterite laser tunable within a wavelength range of 1180-1350 nm.

Two nonlinear optical crystals ($AgGaS_2$) with the same dimensions and the same cutting angles were used for DFG. The height, width, and length of the $AgGaS_2$ crystals were 9, 12, and 24 mm, respectively. Figure 1 showed the tunable MIR laser optical setup.

2) Animals and Preparations: We used WHHLMI rabbits (an animal model for spontaneous hypercholesterolemia, coronary atherosclerosis, and myocardial infarction) [7,8] as an atherosclerotic model and Japan white (JW) rabbits as a normal model. WHHLMI rabbits (females, 24 months old) were provided from Institute for Experimental Animals, Kobe University School of Medicine. Normal and atherosclerotic rabbits were sacrificed by an intravenous injection pentobarbital sodium (50 mg/kg) (Nembutal, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan). Thoracic aortas of them were removed and rinsed with saline. This study was carried out according to the Guideline of Animal Experimentation of Osaka University.

3) Histological Analysis: After laser irradiation, the samples were imbedded by Tissue-Tek O.C.T. Compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan). The frozen imbedded samples were sliced by using a cryotome (Leica CM-1850, Leica Microsystems GmbH, Wetzlar, Germany) for histology. Sections were cut vertically to the tissue surface at 10 μm intervals and mounted on glass slides. The sections

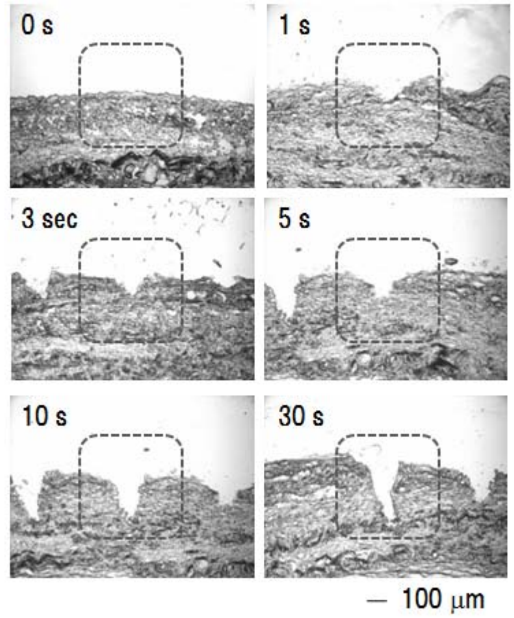

Fig. 2. Histological observation of WHHLMI rabbit thoracic aortas after laser irradiations (wavelength: 5.75 μm, average power density: 80 W/cm2, irradiation time: 0-30 s).

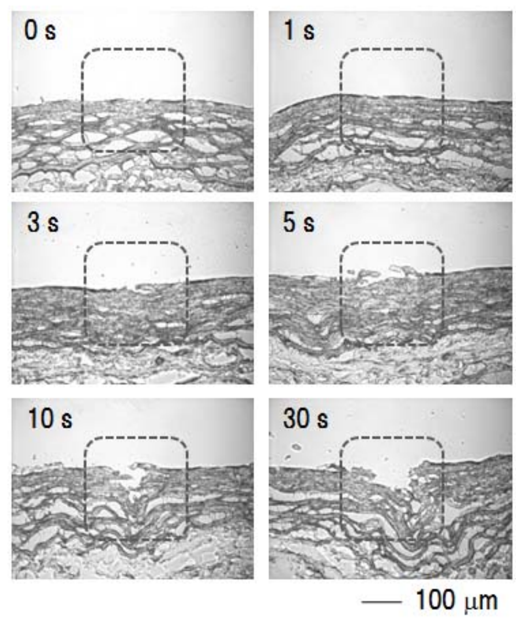

Fig. 3. Histological observation of Japanese white rabbit thoracic aortas after laser irradiations (wavelength: 5.75 μm, average power d it 80 W/ 2 i di ti ti 0 30)

with the crater by laser irradiations were photographed using an optical microscope (DM IRBE, Leica Microsystems) with a cooled color CCD camera (Nebula QICAM, Q Image) and the depths of the crater were measured by Photoshop 5.0.

C. *Result*

Figure 2 showed the histological observation of atherosclerotic thoracic aorta in a wet condition after laser irradiations with the wavelength of 5.75 μm, the irradiation time of 0-30 s and the average power densities of 80 W/cm^2. Ablation was observed at 1 s and higher irradiation time. Ablation depth in 1 s, 3 s, 5 s, 10 s and 30 s were 102±14 μm, 101±15 μm, 108±40 μm, 140±20 μm and 325±53 μm, respectively (n=10, SD).

Figure 3 showed the histological observation of normal thoracic aorta in a wet condition after laser irradiations with the wavelength of 5.75 μm, the irradiation time of 0-30 s and the average power densities of 80 W/cm^2. Ablation was not observed under 1 s and started to perforate the tunica intima and media of normal thoracic aorta over 3 s. Ablation depth in 1 s, 3 s, 5 s, 10 s and 30 s were 0 μm, 11±7 μm, 21±3 μm, 44±21 μm, 67±26 μm, respectively (n=10, SD).

D. Summary

The objective of this study was to demonstrate the effectiveness of nanosecond pulsed laser at 5.75 μm irradiation to atherosclerotic tunica intima. *In-vitro* study using rabbit artery in a wet condition showed that the irradiation of nanosecond pulsed laser with the wavelength of 5.75 μm could remove an atherosclerotic tunica intima effectively. However, the irradiation effect to a normal tunica intima by the wavelength of 5.75 μm was small. It was confirmed that less-invasive interaction to normal thoracic aortas could be induced by the parameters; the wavelength of 5.75 μm, the average power densities of 60-80 W/cm^2 and the irradiation time under 30 s.

In this study, we used a short pulsed laser with a pulse width of 5 ns, which is shorter than the thermal relaxation time of atherosclerotic tunica intima used in this study. HE staining results suggest that the nanosecond pulsed laser with a pulsewidth of 5 ns induce less-thermal effect, mainly mechanical effect (data is not shown). This study shows that nanosecond pulsed laser irradiations with the wavelength of 5.75 μm provide an alternative laser light source as a selective and less-invasive cutting tool for removal of atherosclerotic plaques.

III. Endoscopic Infrared Sperctroscopy Using a Mid-Infrared Tunable Pulsed Laser

A. Background

Since there are many characteristic absorption lines due to molecular vibrations in the mid-infrared (MIR) wavelength range, this wavelength range is often termed the molecular fingerprint region. By using these characteristic absorption lines, MIR spectroscopy is frequently applied to component analysis and structural analysis. In addition, selective excitation and dissociation of molecules are also possible by using tunable MIR lasers. Therefore, non-destructive diagnostics and less- invasive treatments of diseases such as atherosclerosis and gallstones are possible using tunable MIR lasers.

The Fourier transform infrared spectrometer (FT-IR) is one of the most frequently used spectrometer in the MIR wavelength range. In recent years, it is also applied to the analysis of remote samples by combining attenuated total reflection (ATR) method with optical fibers. However, the types of the optical fiber are limited in the MIR wavelength range and the transmittance of the optical fibers for the MIR rays is low compared with those for the visible and near-infrared rays. Since the brightness of the light source used in FT-IR is low, the signal intensity becomes weak with a long optical fiber and the diameter of the ATR probe is too large to introduce into a human body. So far, types of high-power laser sources tunable in the MIR wavelength range have also been restricted. Although free electron lasers (FELs) have been developed as high-power MIR tunable laser sources, very large-size and expensive equipments have been limited the application fields of FELs.

In recent years, hollow optical fibers which can transmit MIR rays have been developed [9,10]. In addition, a tabletop tunable MIR laser source using difference-frequency generation (MIR-DFG laser) has recently been developed. The MIR-DFG laser is tunable within a wavelength range of 5.5–10 μm and generates laser pulses with an energy over 1 mJ/pulse. By using the hollow optical fibers and MIR-DFG laser, higher signal intensity is expected compared with FT-IR, and selective and safe treatments are considered to be possible.

In this chapter, the present status of our development of a system for the endoscopic diagnosis using the tunable MIR laser, and preliminary results obtained with the system are reported.

B. Experimental Setup

1) Tunable MIR-DFG laser: This experiment was carried out by the tunable MIR-DFG laser described in chapter II-B.

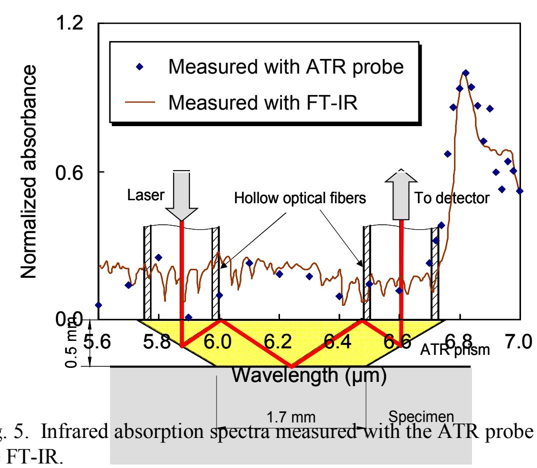

Fig. 5. Infrared absorption spectra measured with the ATR probe and the FT-IR.

Fig. 4. Schematic drawing of the ATR probe.

2) ATR Probe with Hollow Optical Fibers: Since the diameter of commercial ATR probes is too large (> 10 mm) to introduce into a human body, we have designed and manufactured an ATR probe as schematically shown in Figure 4. The ATR prism was made of diamond which is chemically stable and not harmful in a human body. The type I b diamond which had weak internal absorption within the wavelength ranges of about 4–6 μm and 7–15 μm was used.

The inner and outer diameters of the hollow optical fibers were 700 μm and 850 μm, respectively, and the length of the fibers was 1–2 m. The hollow optical fibers can also transmit a visible laser as a guide laser. The laser energy came back from the diamond prism was measured with a laser energy meter with a lower detection limit energy of 0.2 μJ (PE9, Ophir Optronics, Israel).

C. Result

Figure 5 showed the comparison of absorption spectrum of cholesterol measured with the ATR probe by scanning the wavelength of the MIR-DFG laser and that measured with an FT-IR. Two spectral patterns were in good agreement as shown in Fig. 5. In both spectra, a strong absorption peak caused by the C–H bending vibration was observed at the wavelength of 6.83 μm. It is supposed that the combination of the ATR probe and a tunable MIR laser is useful for the diagnostics with the MIR spectroscopy.

D. Summary

We have developed a system for non-destructive and less-invasive diagnosis with the ATR spectroscopy. The absorption spectra of cholesterol was measured with the ATR probe by scanning the wavelength of the tunable MIR laser, and it was in good agreement with that measured with the FT-IR. This catheter-based ATR spectroscopic technique using a high peak power pulsed laser realizes the non-labeling chemical diagnosis inside the body. Especially the applications combined with gastric endoscopy and vessel endoscopy are prospective.

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