Irradiation Effects on Cholesteryl Ester and Porcine Thoracic Aorta of Quantum Cascade Laser in 5.7-μm Wavelength Range for Less-invasive Laser Angioplasty

Keisuke Hashimura, *, # Katsunori Ishii, * Naota Akikusa, ** Tadataka Edamura, ** Harumasa Yoshida, ** Kunio Awazu, ***, †

Abstract We evaluated the potential of using a compact, high-power quantum cascade laser (QCL) in the 5.7-μm wavelength range for less-invasive laser angioplasty, by observing the effects of QCL irradiation on cholesteryl oleate thin films and a porcine thoracic aorta. We compared the results obtained using QCL with those obtained using nanosecond pulsed laser by difference-frequency generation (DFG laser) at a wavelength of 5.75 μm. The QCL irradiation melted the cholesteryl oleate thin films after irradiation for 5-30 s at an average power density of 40 W/cm². On the other hand, the porcine thoracic aorta was not damaged after irradiation for 1-5 s at 40-50 W/cm². This result demonstrates that QCL selectively react with cholesteryl oleate without damaging the porcine thoracic aorta. The QCL induced more thermal damage than the DFG laser under irradiation conditions that gave comparable ablation depths. This finding demonstrates the potential of achieving less-invasive, selective treatment of atherosclerotic plaques using QCL in the 5.7-μm wavelength range, although the pulse structure of the QCL requires improvement to prevent thermal damage to a normal artery.

Keywords: quantum cascade laser (QCL), atherosclerosis, laser angioplasty, less-invasive treatment, cholesteryl ester.


1. Introduction

Laser angioplasty is a treatment modality for atherosclerosis. Unlike percutaneous transluminal coronary angioplasty that uses balloons and stents, laser angioplasty actively removes atherosclerotic lesions. In addition, it is suitable for difficult-to-treat lesions such as totally occluded and complex lesions. However, excessive irradiation by conventional XeCl excimer lasers (wavelength: 308 nm) may damage normal vessels [1]. Therefore, safer lasers are required to accomplish less-invasive laser angioplasty.

Atherosclerotic plaques consist mainly of cholesteryl esters. Radiation at a wavelength of 5.75 μm is strongly absorbed by the C=O stretching mode of cholesteryl esters. Awazu et al. [2] and Fukami & Awazu [3] reported the possibility of selective treatment of atherosclerosis using free electron laser (FEL) at a wavelength of 5.75 μm. Ishii et al. [4] achieved cutting differences between normal tunica intima of an artery and atherosclerotic lesions using nanosecond pulsed laser by difference-frequency generation (DFG laser). These studies demonstrate that it may be possible to achieve less-invasive laser angioplasty using an irradiation wavelength of 5.75 μm. However, DFG laser is too large for clinical use.

A compact laser is required to apply this technique to clinical treatment. Quantum cascade lasers (QCL) are recently developed semiconductor lasers that can emit radiation in the mid-infrared wavelength range [5], and are sufficiently compact for clinical applications. In addition, high output powers have become available recently [6]. They are thus promising light sources for clinical practice.

However, there are concerns about the suitability of the pulse structures generated by DFG laser and QCL. The pulses produced by these two lasers have different widths and repetition rates. It is thus important to determine the different effects of radiation from these two pulsed lasers on atherosclerotic plaques.

The objective of this study was to investigate the potential of using QCL at the 5.7-μm wavelength range for atherosclerosis treatment. The effects of QCL irradiation on cholesteryl oleate thin films were compared with those of DFG laser irradiation. In addition, the effects of QCL irradiation of a porcine thoracic aorta were evaluated.
2. Materials and Methods

2.1 Samples
Cholesteryl oleate thin films, which were used as a model for atherosclerotic plaque, were prepared by dropping 10 μl of 40 mg/ml cholesteryl oleate (C9253, Sigma-Aldrich, USA) in carbon tetrachloride (05-1595, Sigma-Aldrich, USA) on a glass slide or on barium fluoride (BaF2) substrate, and drying at room temperature. Samples on glass slide and BaF2 substrate were used in laser irradiation experiments and mid-infrared absorption spectroscopy measurements, respectively.

The absorption spectra were measured using a Fourier transform infrared spectrometer (MB3000, ABB, Switzerland) coupled with an infrared microscope (μMax, Pike Technologies, USA). The thin film thicknesses were measured using a confocal laser scanning microscope (LEXT OLS3100, Olympus, Japan).

A porcine thoracic aorta was purchased from Medical Device Development Center (MEDDEC, Kobe, Japan). The aorta was resected from a 3.5-month-old pig at MEDDEC. In this study, the aorta was cut into pieces of approximately 5 × 5 mm². To regulate the water content of the samples, the sample surface was wiped gently with clean tissue and 0.1 ml of saline was injected around the sample. For measuring the absorption spectra of the porcine thoracic aorta, the samples were sliced to a thickness of approximately 10 μm using a freezing microtome (CM 1850, Leica Microsystems Co. Ltd., Germany).

From the measured absorption spectra and thicknesses of the cholesteryl oleate thin films and the porcine thoracic aorta, absorption coefficients μₐ and optical penetration depths δₚ (= 1/μₐ) were calculated by Lambert’s law.

2.2 Light Sources
The QCL at 5.7-μm wavelength range was manufactured by Hamamatsu Photonics, K.K., Japan. It generates pulses by injecting a square wave current into the laser element. The pulse width and pulse repetition rate can be controlled. In this study, the pulse width and pulse repetition rate were set at 500 ns and 1000 kHz, respectively. The current injected into the laser element was 1.0 A. Figure 1 shows the output wavelength spectrum obtained under these conditions. The spectrum extends over the wavelength range 5.57-5.92 μm, with maximum intensity at a wavelength of 5.74 μm. The size of this QCL oscillator was 7 cm (width) × 14 cm (depth) × 22 cm (height).

The DFG laser used for comparison was developed by RIKEN, and Kawasaki Heavy Industries, Ltd., Japan [7]. It has a tunable wavelength range of 5.5-10 μm, a pulse width of 5 ns, and a pulse repetition rate of 10 Hz. Mid-infrared output was obtained by inserting two AgGaS₂ crystals between a Q-switched Nd: YAG laser (Tempest 300, New Wave Research Inc., USA) and its wavelength was tuned by rotating the rear mirror of the optical resonator. In this study, the DFG laser wavelength was set at 5.75 μm. The size of this DFG laser optical setup was 97 cm (width) × 147 cm (depth) × 28 cm (height).

2.3 Irradiation Setup
Figure 2 shows the optical setup used for QCL and DFG laser irradiation. First, cholesteryl oleate thin films were irradiated by the two lasers. The beam sizes for QCL and DFG laser were 240 × 320 μm and 140 × 200 μm, respectively. The average power density and irradiation time were varied in the ranges of 20-100 W/cm² and 1-30 s, respectively for QCL irradiation, and in the ranges of 5-30 W/cm² and 1-30 s, respectively, for DFG laser.

Next, the porcine thoracic aorta was irradiated by the QCL. Spot irradiation (beam size: 250 × 290 μm) was performed for surface observations, while line irradiation laser was pumped by a Q-switched Nd: YAG laser (Tempest 300, New Wave Research Inc., USA) and its wavelength was tuned by rotating the rear mirror of the optical resonator. In this study, the DFG laser wavelength was set at 5.75 μm. The size of this DFG laser optical setup was 97 cm (width) × 147 cm (depth) × 28 cm (height).
(beam size: 240 × 330 μm) was performed for cross-sectional observations. The average power density and irradiation time were varied in the ranges of 100–300 W/cm² and 1–30 s, respectively, for spot irradiation, and in the ranges of 50–250 W/cm² and 1–30 s, respectively for line irradiation. A motorized stage moved linearly at a constant rate was used. The scanning speed was calculated by dividing the beam size (330 μm) by the irradiation time per spot (1–30 s).

Finally, the porcine thoracic aorta was irradiated by the DFG laser to compare the thermal damage width with that by the QCL. Linear irradiation was performed to measure the ablation depth and the thermal damage width by cross-sectional observation. The average power density was 40 W/cm² and the irradiation time was 10 s.

Table 1 summarizes the experimental conditions of the QCL and the DFG laser.

<table>
<thead>
<tr>
<th>Laser</th>
<th>Pulse width [ns]</th>
<th>Pulse repetition rate [Hz]</th>
<th>Irradiation time [s]</th>
<th>Average power density [W/cm²]</th>
<th>Peak power density [W/cm²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCL</td>
<td>500</td>
<td>1 × 10⁶</td>
<td>1–30</td>
<td>20–300</td>
<td>40–600</td>
</tr>
<tr>
<td>DFG laser</td>
<td>5</td>
<td>10</td>
<td>1–30</td>
<td>5–40</td>
<td>1 × 10⁶–8 × 10⁶</td>
</tr>
</tbody>
</table>

2.4 Observation

The changes induced by QCL and DFG laser irradiation in cholesteryl oleate thin films were observed and movies were acquired using a CCD camera.

After irradiating the porcine thoracic aorta, the surfaces were observed under an optical microscope to evaluate carbonization and coagulation. After line irradiation, the samples were frozen, sliced at a thickness of about 3 μm using a freezing microtome and stained with hematoxylin and eosin (HE) staining. Coagulation, carbonization and vaporization of the sample surface were evaluated by microscopic observations of the HE-stained samples. In addition, the depth of ablation and depth of thermal damage were measured from cross-sectional images.

3. Results

3.1 Absorption Coefficients and Spectra of Samples

Figure 3 shows the absorption spectra of cholesteryl oleate thin films and porcine thoracic aorta. For the cholesteryl oleate thin films, the average thickness was 9.3 ± 2.0 μm (n = 12), and the average density was 6.8 ± 1.2 μg/mm³. The maximum μa was (1.9 ± 0.8) × 10⁴ cm⁻¹ at the wavelength of 5.75 μm. The δp was calculated to be 5.4–39 μm for the wavelength range of 5.6–5.9 μm. For the porcine thoracic aorta, the maximum μa was (1.2 ± 0.5) × 10⁴ cm⁻¹ at the wavelength of 6.05 μm. The δp was 18–62 μm for the wavelength range of 5.6–5.9 μm.

3.2 Irradiation of Cholesteryl Oleate Thin Film

Figure 4 shows the effects of QCL irradiation of
cholesteryl oleate thin films. No changes were observed at average power densities of 20 and 30 W/cm\(^2\). At an average power density of 40 W/cm\(^2\), melting of cholesteryl oleate was observed after irradiation for 5 s. At 50 W/cm\(^2\) and above, melting occurred within 1 s.

Figure 5 shows the effects of DFG laser irradiation of cholesteryl oleate thin films. At 30 W/cm\(^2\) and below, changes were induced within 1 s. The thin films were mechanically ablated by DFG laser irradiation. Ablation debris was observed during irradiation.

### 3.3 Irradiation of Porcine Thoracic Aorta

Figure 6 shows the surface views of a QCL spot-irradiated porcine thoracic aorta sample. At 100 and 150 W/cm\(^2\), coagulation was observed after irradiation for 5 s and longer. At 200 W/cm\(^2\), coagulation was observed after 1 s and longer and carbonization was observed after 5 s and longer. At 250 and 300 W/cm\(^2\), carbonization was observed after 1 s and longer. The area of carbonization increased with increasing irradiation energy.

Figure 7 shows the cross-sectional views of a QCL line-irradiated porcine thoracic aorta. At 40 and 50 W/cm\(^2\), coagulation was observed after irradiation for 10 s and longer. Shrinkage was observed after 10 s and longer, and found to be due to coagulation. At 100 W/cm\(^2\), shrinkage was observed after 1 s and longer. At 150 W/cm\(^2\), intermittent carbonization and ablation were observed after 3, 5, and 10 s. Continuous carbonization and ablation were observed after 30 s. At 200 W/cm\(^2\), intermittent carbonization and ablation were observed after 1 s, and continuous carbonization and ablation were observed after 5 s and longer. At 250 W/cm\(^2\), continuous carbonization and ablation were observed after 1 s and longer.

Figure 8 shows the relationship between the QCL irradiation time and the ablation depth of porcine thoracic aorta \((n = 10)\). The ablation depth increased with increasing average power density. The increase in ablation depth became attenuated as the irradiation time increased.
3.4 Comparison of Thermal Damage Width

Figure 9 shows the ablation depths and thermal damage widths produced by QCL and DFG laser irradiation of porcine thoracic aorta. The QCL and DFG laser had average power densities of 200 and 40 W/cm², respectively. The irradiation time was 10 s for both lasers. The QCL and DFG laser respectively produced ablation depths of 133 ± 36 μm and 119 ± 18 μm, and thermal damage widths of 156 ± 27 μm and 16 ± 6 μm. These results demonstrate that QCL induced greater thermal damage than the DFG laser under irradiation conditions that produce comparable ablation depths.

4. Discussion

4.1 Interaction between Lasers and Cholesteryl Oleate

The average thickness of the cholesteryl oleate thin films was 9.3 ± 2.0 μm. In DFG laser irradiation, δp was 5.4 μm because the DFG laser was set at a wavelength of 5.75 μm and the output wavelength spectrum is narrow. In contrast, QCL emits in the wavelength range of 5.6-5.9 μm. Thus, δp was 5.4-39 μm in QCL irradiation. Therefore, QCL probably interacted with the films at a greater depth compared with DFG laser.

Laser-tissue interactions are determined by the interaction time τint and peak power density. To irradiate cholesteryl oleate thin films, the average power densities of QCL and DFG laser were 20-100 W/cm² and 5-30 W/cm², respectively, corresponding to peak power densities of 40-200 W/cm² and 100-600 MW/cm², respectively.

The τint is determined by the relationship between pulse interval and relaxation time. The thermal relaxation time τtherm and the stress relaxation time τstress are given by

\[ \tau_{\text{therm}} = \frac{\delta p^2}{4\alpha \Delta T} \]  

\[ \tau_{\text{stress}} = \frac{\delta p}{c_s \mu a c_s} \]  

where α is the thermal diffusivity and c_s is the speed of sound. And, α is defined as the ratio of thermal conductivity L to the product of density ρ and specific heat cs. Since L, ρ, and cs of cholesteryl oleate are respectively 3.5 \times 10^{-3} W/(K cm), 1.052 g/cm³, and 1.6 J/(g K) [9-13], α was calculated to be 2.1 \times 10^{-3} cm²/s. And cs of cholesteryl oleate was taken to be 1.5 \times 10³ m/s based on the speed of sound in lipid (1.5 \times 10³ m/s). Therefore, τtherm and τstress of cholesteryl oleate were calculated to be 35-1800 μs and 3.6-29 ns, respectively, for the wavelength range of 5.6-5.9 μm (output wavelength range of the QCL). Both τtherm and τstress are minimum at the wavelength of 5.75 μm.

The pulse interval of QCL (500 ns) is shorter than τtherm for cholesteryl oleate. Thus, τint corresponds to the irradiation time (1-30 s). The QCL thus acts as a quasi-continuous wave laser for cholesteryl oleate thin films and induces photothermal interaction. In photothermal interaction, the energy of laser irradiation is transferred into heat. According to the degree of heating, thermal effects (such as melting, carbonization and vaporization) can be achieved.

In contrast, the pulse interval of the DFG laser (0.1 s) is longer than τtherm for cholesteryl oleate. Thus, τint corresponds to the pulse width (5 ns). Because τint is comparable to τstress, photomechanical interaction is induced. In photomechanical interactions, the stress wave with velocity c_s cannot leave the heated volume during the laser pulse, and ablation of the material can be achieved while the surrounding is slightly damaged.

In addition, FEL has been reported to induce mechanical and slight thermal interaction in cholesteryl oleate [2,3]. Because the present FEL has a pulse interval of 0.1 s, τint corresponds to the pulse width (15 μs). And, τint is longer than τstress but shorter than τtherm.

We thus hypothesize that QCL, DFG laser, and FEL induce different interactions in cholesteryl oleate. However, this hypothesis is based on the density of the cholesteryl oleate films used in this study. To investigate the interactions between real atherosclerotic lesions and laser radiation, the absorption coefficient of arterial lesions at the 5.7-μm wavelength range should be determined accurately.

4.2 Potential of Less-invasive Treatment by QCL

Cross-sectional observations of the porcine thoracic aorta linearly irradiated by QCL revealed that non-invasive irradiation parameters were obtained for irradiation time of 5 s or shorter at average power densities of 40 and 50 W/cm². On the other hand, QCL irradiation of cholesteryl oleate thin films caused melting after irradiation for 5 s or longer at an average power density of 40 W/cm², and after 1 s or longer at 50 W/cm² (Fig. 10). These results indicate the potential of utilizing QCL for less-invasive treatment of atherosclerotic plaque because it melts cholesteryl oleate thin films without damaging normal vessels.

The QCL used in this study induced photothermal interactions in the porcine thoracic aorta. Because the melting point of cholesteryl oleate is 44–47°C at atmo-
Fig. 10  Effects of QCL irradiation of cholester-yl oleate thin films and porcine thoracic aorta.

spheric pressure, which is lower than the thermal denaturation temperature of normal vessels (around 60°C), selective reactions were considered to have been realized.

The water content of samples greatly influences the effect of QCL irradiation, since water has a relatively high absorption coefficient at mid-infrared wavelengths. In the wavelength range of 5.6–5.9 μm, the absorption coefficient of the samples increases and the optical penetration depth decreases with increasing water content. In addition, water has a higher heat capacity than protein. Hence, thermal effects in the sample decrease with increasing water content.

Consequently, at water contents similar to those of intravascular tissue, a higher energy density is required to induce the same irradiation effects observed in the present experiment. This implies that the maximum energy density that does not damage normal vessels and the lowest energy density that can remove atherosclerotic plaque both increase.

The presence of water reduces thermal effects, but when the critical water temperature (374°C) is reached, strong ablation that is difficult to control occurs. As discussed above, the presence of water has both beneficial and detrimental effects. More detailed studies are required to investigate the influence of water and to determine optimal optical irradiation conditions.

Comparison of the thermal damage widths produced by the two lasers reveals that the QCL induced more thermal damage than the DFG laser for comparable ablation depths. To prevent thermal damage, the pulse structure produced by the QCL needs to be improved. In this study, the QCL had a pulse interval of 500 ns, which is too short for thermal diffusion to occur. Therefore, a pulse interval longer than the thermal relaxation time of the porcine thoracic aorta should be used.

5. Conclusion

A QCL in the 5.7-μm wavelength range selectively reacted with cholesteryl oleate without inducing thermal damage to porcine thoracic aorta. This finding demonstrates that QCL has the potential to be used in less-invasive and selective laser angioplasty. The QCL generated a quasi-continuous wave and induced photothermal interactions in both cholesteryl oleate and porcine thoracic aorta. Thus, the QCL induced greater thermal damage in the porcine thoracic aorta compared to the DFG laser. To prevent thermal damage to a normal artery, it is necessary to improve the QCL pulse structure.

Acknowledgement

This work was supported by JSPS KAKENHI Grant Number 24241209.

References

Keisuke Hashimura
Keisuke Hashimura received his Bachelor’s degree in engineering from School of Engineering, Osaka University, Japan in 2012. He currently investigates the laser-tissue interactions for novel laser surgery and medicine in Medical Beam Physics Laboratory, Graduate School of Engineering, Osaka University, Japan. He received a best research award of JBMES 2012.

Katsunori Ishii
Katsunori Ishii received his Ph.D. degree in engineering from Graduate School of Engineering, Osaka University, Japan in 2007. Since 2008, he has been an assistant professor in Medical Beam Physics Laboratory (Professor Kunio Awazu), Graduate School of Engineering, Osaka University, Japan. His research interest is the basics in laser surgery and medicine including laser-tissue interaction, tissue optics and medical laser applications. He is a lifetime member of SPIE.

Naota Akikusa
Naota Akikusa received his Bachelor’s degree in engineering from Graduate School of Engineering, Hokkaido University, Japan in March 1996. He entered Hamamatsu Photonics K.K., Japan in April 1996. Since 2010, he has been in Development Bureau Laser Device R&D Group, Hamamatsu Photonics K.K., Japan. His research interest is the development of mid-infrared lasers and devices.

Tadataka Edamura
Tadataka Edamura received his Ph.D. degree in engineering from Graduate School of Science and Technology, Keio University, Japan in March 1995. He entered Hamamatsu Photonics K.K., Japan in April 1995. He is a senior researcher of Material Research Group, Central Research Laboratory, Hamamatsu Photonics K.K., Japan. His research interests are the optical physics of semiconductor quantum structures and applications of light-emitting devices. He is a member of IEEE.

Harumasa Yoshida
Harumasa Yoshida received the B.S. degrees in electrical engineering, the M.S. degrees electrical and electronic engineering, and the Ph.D. degree in materials science from Mie University, Japan, in 1980, 1982, and 2003 respectively. He joined Brother Industries, Ltd., Nagoya, Japan in 1982, where he was engaged in the research and development of robotics devices. In 1985, he joined Hamamatsu Photonics K.K., Shizuoka, Japan, and has engaged in research and development of photonic devices and their applications. Since 2004, he has involved in the research on nitride material and development of ultraviolet laser diodes. Currently, he leads a research and development section of laser devices. Dr. Yoshida is a member of the Japan Society of Applied Physics, the Institute of Electrical and Electronics Engineers, and the International Society for Optical Engineering.

Kunio Awazu
Kunio Awazu received his Master’s degree and Ph.D. degree of engineering from Graduate School of Engineering, Kobe University, Japan in 1984 and 1996, respectively. He received Dr. Med. Sci. degree from Juntendo University, Japan in 1997. Since 2005, he has been a professor in Medical Beam Physics Laboratory, Graduate School of Engineering, Osaka University. He has also been a professor in The Center for Advanced Medical Engineering and Informatics, and Graduate School of Frontier Biosciences, Osaka University since 2007 and 2011, respectively. His research interests are tissue optics, medical laser applications, photomedicine and photobiology, and their regulatory sciences for medical device developments. He is a country representative of The World Federation of Laser Dentistry, and a lifetime member of SPIE.