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The Effect of an Optical Clearing Agent on Tissue Prior to 1064-nm Laser Therapy

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Background and Objectives

Although lasers have been widely applied in tissue treatment, the light penetration depth in tissues is limited by the tissue turbidity and affected by its absorption and scattering characteristics. This study investigated the effect of using an optical clearing agent (OCA) on tissue to improve the therapeutic effect of 1064 nm wavelength laser light by reducing the heat generated on the skin surface and increasing the penetration depth.

Materials and Methods

A diode laser (λ = 1064 nm) was applied to a porcine specimen with and without OCA to investigate the penetration depth of the laser light and temperature distribution. A numerical simulation using the finite element method was performed to investigate the temperature distribution of the specimen compared to *ex-vivo* experiments using a thermocouple and double-integrating sphere to measure the temperature profile and optical properties of the tissue, respectively.

Results

Simulation results showed a decrease in tissue surface temperature with increased penetration depth when the OCA was applied. Furthermore, both absorption and scattering coefficients decreased with the application of OCA. In *ex-vivo* experiments, temperatures decreased for the tissue surface and the fat layer with the OCA, but not for the muscle layer.

Conclusion

The use of an OCA may be helpful for reducing surface heat generation and enhance the light penetration depth in various near-infrared laser treatments.

Key words

Laser therapy; Optical clearing agent; Finite element method; Tissue optical property; Temperature distribution

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INTRODUCTION

Recently, near-infrared laser is used to permeate optical energy deep into tissues to induce photochemical action and photothermal actions, induce metabolic activity of muscle fibers and surrounding tissues, relieve pain and inflammation, and treat thrombolysis.[1-4] Typically and frequently used lasers in near-infrared laser treatment include Nd:YAG (Neodymium-doped yttrium aluminum garnet) lasers and diode lasers with a wavelength of 1064 nm. It has been reported that while the Nd:YAG laser shows a high therapeutic effect, the diode laser of the same wavelength is cheaper than the Nd:YAG laser with reduced cost and high efficiency.[5,6] Although the length of a diode laser coherence is shorter than that of a Nd:YAG laser coherence, much absorption occurs from the outer skin, thus limiting the transmission depth of light energy. Due to such restriction, many patients complain of fever on the surface of the skin during a high-power treatment with a diode laser.[1-6] To solve such problem, the objecteive of this study was to determine whether the treatment efficiency of a diode laser could be improved by using an optical clearing agent (OCA). Research is being actively conducted to transmit light deeply to living tissues in treatment using a laser. Various methods such as micro-needle, microwave, and micro-current are used to improve the transmission of laser light.[7-9]

The OCA-based method matches the refractive index between cells and temperament in the dermis to reduce the optical scattering phenomenon, thus allowing irradiated light to be transmitted to deeper layers for optical diagnosis and improved treatment performance.[7,9] Although the mechanism for OCA has not been clarified, various hypotheses have been proposed, including tissue dehydration, intercellular or intracellular water formation with refractive index changes and high refractive index components, and structural deformation or dissociation of collagen.[7,9] The light scattering reducing effect of OCA can increase the efficiency of optical diagnosis and treatment by allowing the irradiated light to be transmitted to the depth of the skin tissue.

The simulation with finite element method in this study is often used in various diagnostics and treatments using light these days. Using this method, it is possible to predict the diffusion of columns in a tissue according to the distribution of light and heat distribution by depth.[10,11] In addition, based on simulation results during laser treatment, it is possible to support the setting of laser parameters that are more effective for treatment. This makes it possible to minimize the site of thermal damage and reduce side effects.[10,11] In this study, finite element analysis of changes in heat generation on the surface of the skin was performed and how heat was transferred to deep tissues when OCA was applied to porcine skin during treatment with a 1064 nm diode laser was determined. Simulation was performed and results were compared through ex-vivo experiments.

MATERIALS AND METHODS

This research is approved by Institutional Animal Care and Use Committee (IACUC) in Daegu Catholic University (Approval No. IACUC-2015-036). In this study, 70% glycerol (4066-4405, Daejung Chem. Co., Korea) was used to reduce light scattering in tissues. It is suitable for the human body and has been proven to reduce light scatter-

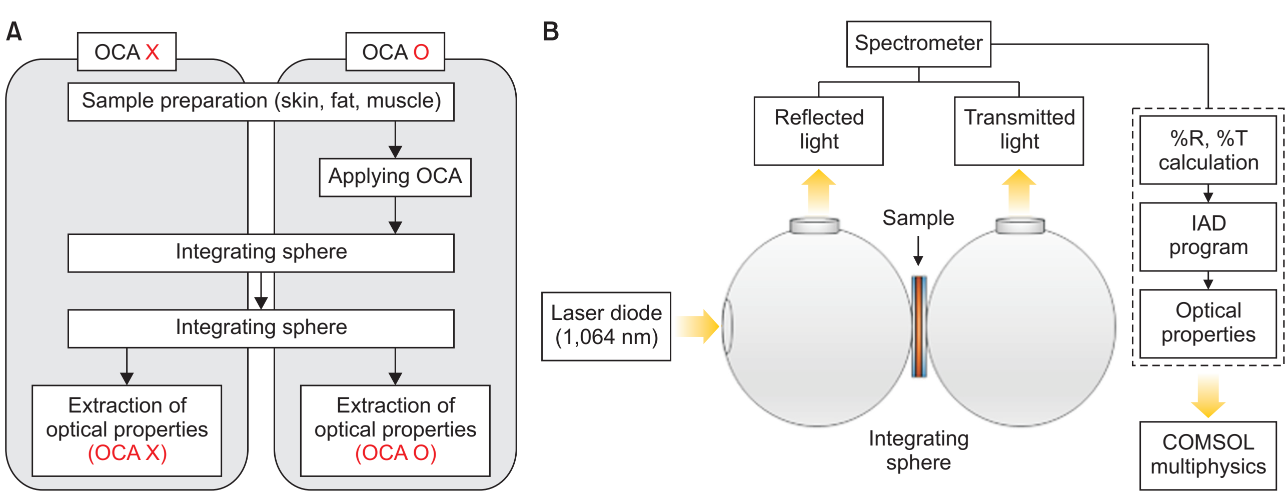

Fig. 1. Ex-vivo experiment layout for optical property measurements: (A) Optical property extraction flowchart for the samples, (B) Double-integrating sphere diagram to measure %R and %T of the samples.

ing.[11,12]

A porcine tissue sample composed of a skin layer, an adipose layer, and a muscle layer was used to observe light characteristics changed by OCA. Double-integrating spheres (AvaSphere-50, Avantes, Inc., USA) were used to measure reflection and transmission spectra before and after application of glycerol to the procine tissue sample. The light source used in the double-integrating sphere experiment was a 1064 nm Diode Laser (LU1064D300-D, Lumics Inc., Germany). Spectral bandwidth information was obtained using a NIR-spectrometer (InGaAs spectrometer, Edmund Optics, USA) (Fig. 1). Measured transmission and reflection spectra were calculated as M_R and M_T respectively, with the following equations.

$$M_R = r_{std} \cdot \frac{R_{sample} - R_{background}}{R_{100\%} - R_{background}}$$
$$M_T = \frac{T_{sample} - T_{background}}{T_{100\%} - T_{background}} \quad (1)$$

At this time, the obtained transmission and reflection spectra were converted into effective optical coefficients via the Inverse Adding-Doubling (IAD) Method.[13,14] IAD is a method for generalizing light scattering and absorption characteristics of a medium. Transmission and reflection spectra measured by Double-integrating spheres could be converted into the sample's optical properties such as the absorption coefficient (μ_a) and the scattering coefficient (μ_s) through the IAD program.[15,16]

The spectrum-intensity weighted average method was used to reflect the accuracy of the Diode laser with a shorter coherence length than the Nd:YAG laser.[16] Optical coefficients within the wavelength width of 1066 nm at 1058 nm, the bandwidth of the spectrum measured using a spectrometer, were converted into generalized intensities corresponding to each wavelength. The effective optical coefficient was calculated by substituting the weight for this into the obtained optical coefficient. Each absorption and scattering coefficient calculated in this way were applied to mathematical modeling.[16]

$$I_{normalized_\lambda} = \frac{I_\lambda}{\sum_{\lambda_0=1066}^{\lambda=1058} I_\lambda}, \quad \sum_{\lambda_0=1066}^{\lambda=1058} I_{normalized_\lambda} = 1 \quad (2)$$
$$\mu a\_tissue\_Diode = \sum_{\lambda_0=1066}^{\lambda=1058} I_{normalized_\lambda} * \mu a\_tissue\_\lambda$$

When irradiating a biological tissue with a diode laser, the heat transfer equation of the finite-element analysis program (COMSOL Multiphysics., Comsol Inc., Burlington, MA, USA) was used to simulate the heat distribution generated before and after applying the OCA.[11,17] The size of the sample used in the simulation was 20 mm in length and 20 mm in width. Heights of the skin, fat and muscle layer were 1.5 mm, 3 mm, and 3 mm, respectively. The laser used was also irradiated for 60 seconds at a beam diameter of 10 mm, a power of 12 W, and a frequency of 50 Hz. The initial temperature of the sample was fixed at 25°C, the temperature of the laboratory environment. The mesh consisted of 3228 domains and 4424 boundar-

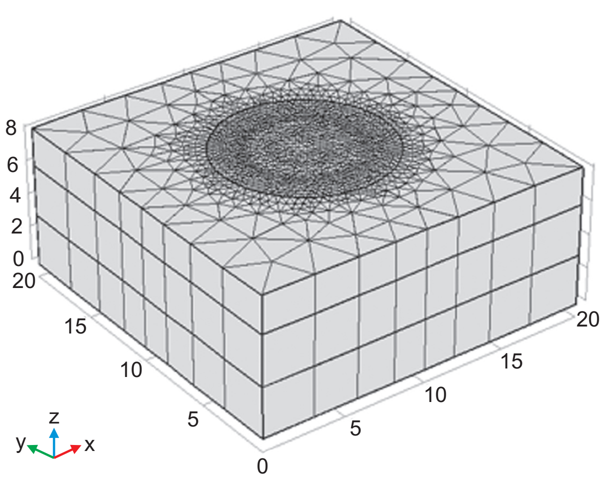

Fig. 2. Mesh structure with three layers (skin, fat, and muscle) in Finite Element Method to compare the simulation results with the ex-vivo experiment results.

Table 1. Material properties of tissues used in the simulation[17]

Property	Skin	Fat	Muscle
Thermal conductivity; *k* [*W*/(*m*·*K*)]	0.21	0.4	0.49
Specific heat; C [*J*/(*kg*·K)]	3181.82	2348	3421
Density; *p* [*kg*/*m*^{3}]	1000	911	1090

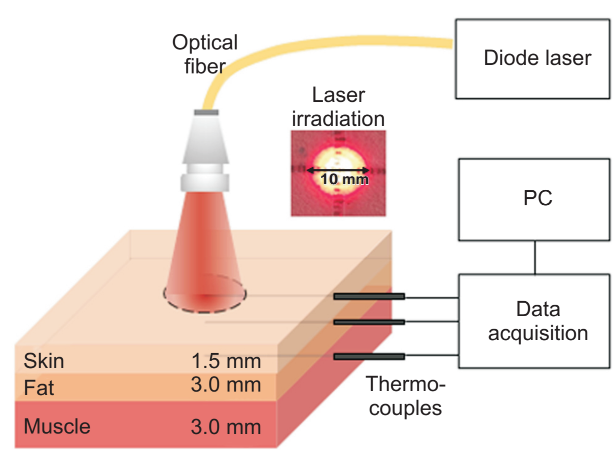

Fig. 3. Layout of ex-vivo temperature measurements using a diode laser and thermocouples.

ies (Fig. 2). Table 1 shows thermodynamic coefficients of each layer used in the simulation.[17]

To compare simulation results with ex-vivo experimental results, porcine specimens before and after OCA application were irradiated with a laser under the same conditions (beam diameter = 10 mm, repitition rate = 50 Hz, exposure time = 60 sec). In addition, three thermocouples (HYPO-1, Omega Eng. Inc., USA) and a data logger (TC-08, Omega Eng. Inc., USA) were used to measure the temperature of each panniculus. Temperature changes were observed in real time (Fig. 3). Each thermocouple was located on the surface of the skin layer, the center of the fat layer, and the center of the muscle layer.

RESULTS

Fig. 4 shows changes in absorption coefficient and scattering coefficient as optical characteristics before and after OCA application depending on the wavelength of the tissue. It was confirmed that the absorption coefficient and the scattering coefficient of the wavelength after OCA application decreased to a certain level. It could be predicted that the light at the time of laser irradiation was deeply transmitted into the tissue.

By substituting the absorption coefficient and the scattering coefficient obtained from Fig. 4 into the simulation, the temperature change of each floor with time (Fig. 5) and the temperature distribution according to the depth of the sample (Figs. 6, 7) were obtained. The temperature change with time decreased by about 6°C as a whole for samples coated with OCA between the skin layer and the fat layer, whereas the temperature difference for the muscle layer was within 0.5°C.

Fig. 8 shows results of irradiating surfaces of five samples not coated with OCA and five samples coated with OCA with a laser. The temperature of each floor was measured for 60 seconds using a thermocouple. After applying OCA to the sample, the temperature of the epi-

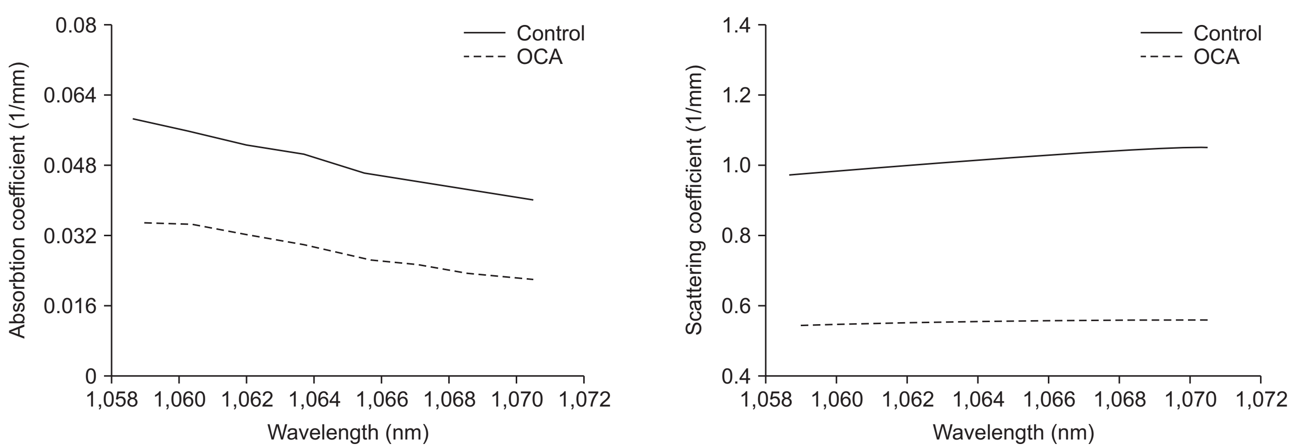

Fig. 4. Absorption coefficients (left) and scattering coefficients (right) in tissues depending on the wavelength.

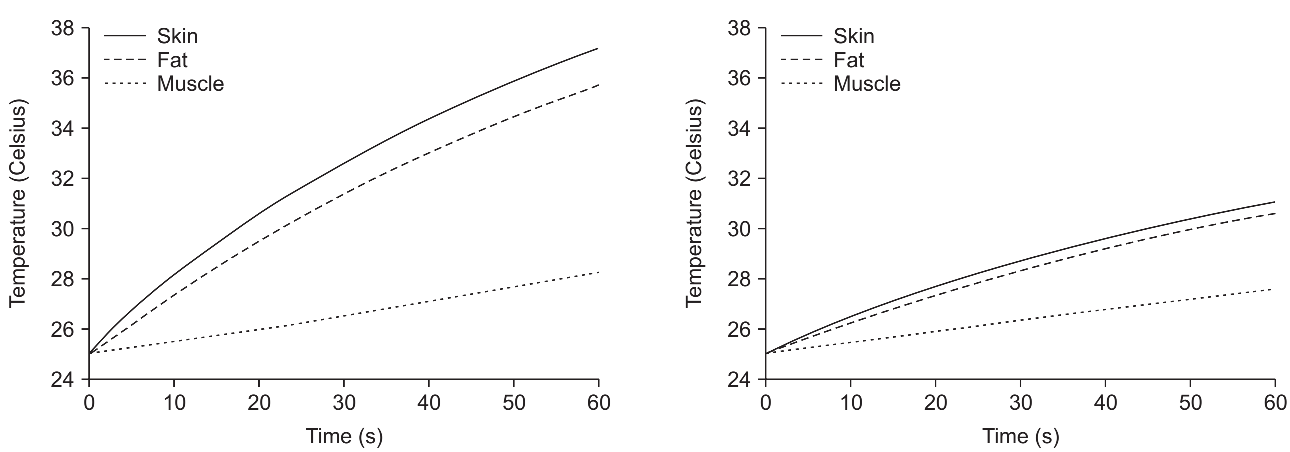

Fig. 5. Simulation results of temperature changes over time with contol (left) and OCA (right).

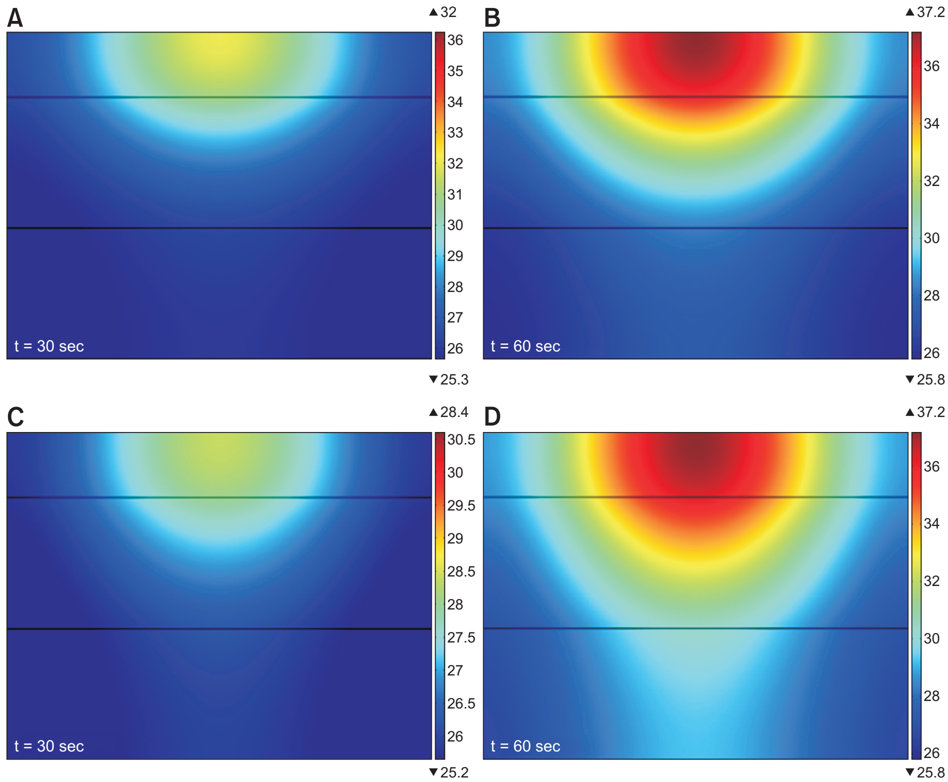

Fig. 6. Simulation results of temperature changes with control (A, B) and OCA (C, D).

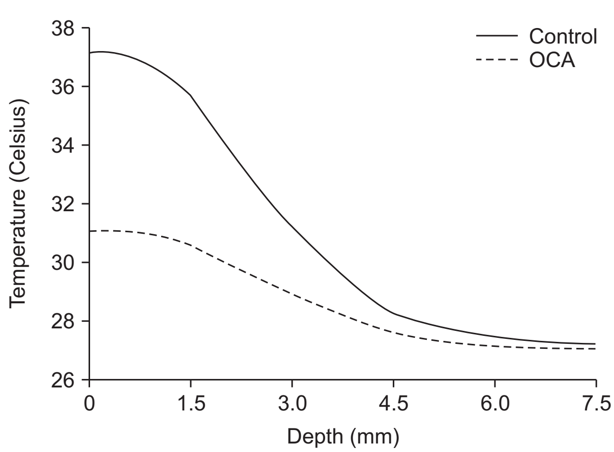

Fig. 7. Simulation results of temperature changes over the depth with control (solid line) and OCA (dotted line).

dermis and the fat layer measured was about 2°C lower than that of the sample without OCA. The temperature of the muscle layer with or without OCA application was the same. From these results, it is considered that when OCA is applied by laser treatment, heat generation on the surface of the skin can be minimized and laser stimulation can be applied deeply into the skin.

DISCUSSION

In laser-based treatment, the depth of light transmission has a great influence on the outcome of treatment. Due to scattering properties of the tissue, the efficiency of treatment can be reduced if the light is not sufficiently transmitted deeply into the tissue. The light transmission depth can be varied by light scattering properties of the tissue and it can be enhanced by using OCA, a light scattering reducing agent. Much research has been done to improve the light transmittance of biological tissues using mechanical, physical, and chemical methods. OCA is not only also widely used for current laser-related diagnosis and treatment, but also used for ultrasound imaging and laser ablation.[9,12,18,19] In this study, we determined whether light scattering reducing substances could increase the treatment efficiency using a 1064 nm diode laser that could be applied to various intracutaneous diseases and musculoskeletal diseases.

Absorption coefficients and scattering coefficients, which indicate optical properties of the tissue, can vary depending on the tissue and environment in which they are used. Therefore, in this study, a more accurate optical coefficient was obtained by direct measurement using a double-integrating sphere and the IAD program. To observe changes in the optical coefficient based on

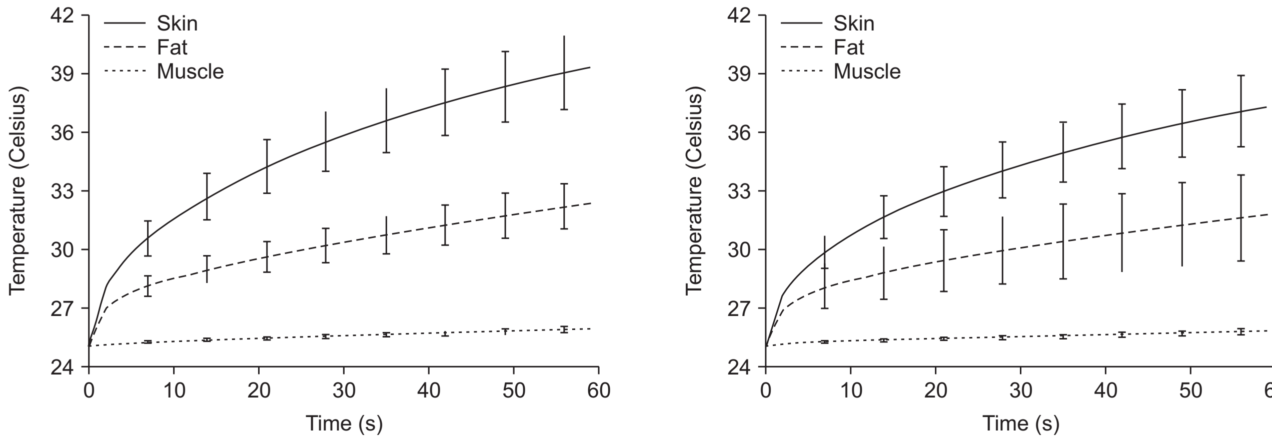

Fig. 8. Ex vivo temperature changes of each layer in tissue with control (left) and OCA (right).

the wavelength value of the light source, the measured optical coefficient was converted using the spectrum-intensity weighted average method with effective absorption coefficient and scattering coefficient according to the wavelength. Results of this study could be used as basic data for comparative analysis with Nd:YAG laser in the same wavelength band in the future. Fig. 4 shows that the OCA-coated sample has a lower absorption coefficient and scattering coefficient over the entire wavelength band than the sample before OCA coating. This proves that the permeation depth of the tissue is enhanced by OCA. In addition, the absorption coefficient of the tissue tended to decrease as a whole as the wavelength became longer, consistent with a previous study showing that the absorption coefficient of water, which occupies more than 70% of the tissue, gradually decreases from 1000 nm to 1100 nm.[20] As a result of predicting heat distribution by substituting the converted optical coefficient into simulation, a phenomenon of lowering the temperature of the skin and the fat layer was observed for the sample coated with OCA as shown in Fig. 5. It could be seen that the light was transmitted to the muscle layer without inducing a temperature rise on the surface of the skin. It could be judged that the reason why the temperature change of the muscle layer, the lowermost part, was not large because heat diffusion by light occurred in all directions as shown in Figs. 6, 7. Results obtained through ex-vivo experiments also showed lower temperature changes in the skin and fatty layers of OCA-coated samples compared to those for non-OCA-coated samples. However, it was judged that the reason why the temperature distribution result with OCA applied was slightly higher than the simulation result was because the temperature at one point from the center of each floor was measured according to the time using a thermocouple.

There can be differences from results of simulating the entire part. The temperature difference might also be affected by not considering how chromophores present in the tissue like melanin, water, and hemoglobin could change the heat generated during laser irradiation. Furthermore, a biological tissue can have the refractive index matching process when OCA is applied, and the morphological changes such as an increase of the tissue background refractive index with a consequent reduction of scattering components can occur. This process might also affect the enhancement of the penetration depth of light in tissue with low heat generation on the skin layer.[19]

Results of simulations and ex-vivo experiments revealed that by applying a light scattering reducing substance during near-infrared diode laser treatment, the heat generated on the surface of the skin was reduced. Due to reduced heat generation, it could be judged that a patient's complaint of pain could be reduced with an increased satisfaction with the treatment. Furthermore, as a result, the laser beam reached the deeper part well to increase the light transmission depth. Thus, a better therapeutic effect can be expected. There is a need for a measure to determine that OCA has been sufficiently absorbed from the surface of the skin and a method for reducing the time required for absorption. Further experiments are needed to determine the efficiency by comparing the light scattering reduction effect through comparison of Nd:YAG laser treatment and diode laser treatment with the same wavelength of 1064 nm.

In this study, we compared and analyzed heat generation and distribution when a skin model with three layers of skin, fat, and muscle was irradiated with a 1064 nm near-infrared diode laser before and after OCA application through simulation and experiment. It was confirmed that the application of an OCA during near-infrared diode laser treatment could reduce heat generation on the skin surface and stimulate the skin deeply.

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