Ultrasound-assisted photothermal therapy and real-time treatment monitoring

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Abstract: Photothermal therapy (PTT) has the capability for selective treatment, in which light delivered to the target is converted into heat and subsequently causes coagulative necrosis. However, optical scattering in biological media limits light penetration, thus reducing therapeutic efficacy. Here, we demonstrate that the temperatures generated by light and ultrasound energies can be added constructively in resected melanoma cancers, which causes an increase in treatment depth. This method is called dual thermal therapy (DTT). It is also shown that combined ultrasound and photoacoustic images acquired using the pulse sequence proposed in this paper can be used for real-time monitoring of DTT.

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1. Introduction

Thermal therapy rests on the fact that coagulative necrosis occurs when delivered energy causes a local temperature rise above 45°C [1]. High-intensity focused ultrasound (HIFU) surgery uses acoustic energy to increase local temperature for tumor cell death [2]. Since the aperture size, focal length, and operating frequency of a HIFU transducer determine an ultrasound (US) focal volume in which localized heating is induced, the location and size of treatment region are relatively easily adjusted [3]. One disadvantage of HIFU surgery is the high possibility of irreversible damage to normal tissues as well as tumors. For this reason, an image-guided method is a pivotal element of HIFU surgery to minimize the adverse side effect [4]. On the other hand, photothermal therapy (PTT) is conducted by transmitting light energy into target absorbers such as melanoma. The incident light energy is converted into heat in the absorbers, thus resulting in coagulative necrosis. Since a local temperature rise occurs only in target absorbers, PTT is capable of selective treatment, i.e., minimal damage to normal tissues unlike HIFU surgery. For optimal PTT, therefore, a light wavelength at which the target chromophore can maximally absorb delivered energy should be used. The problem here is that this requirement is not always met because of optical scattering inversely proportional to the wavelength of light [5]; the shorter the wavelength, the lower the therapeutic depth. One typical example of this problem is melanoma treatment for which light in the second near-infrared (NIR) window in the range of 1000 to 1400 nm is used in clinics to increase light penetration depth although the maximum optical absorbance of melanoma...
occurs at wavelengths between 540 and 570 nm [6,7]. This inevitably leads to a reduction in therapeutic efficacy.

The most viable solution to the problem mentioned above is to use photothermal agents because they can be fabricated to bind into tumor cells and to have a high optical absorbance at a wavelength in the second NIR window [8,9]. This facilitates increased treatment depth and enhanced selective therapy. To this end, various photothermal agents have been intensively developed although the safety of non-biological materials in clinical use still needs to be confirmed [8,10,11]. Another possible solution is to overcome the optical scattering effect itself by either the wavefront-shaping methods [12] or the temporal change of the optical scattering characteristics of a medium to Mie scattering by means of ultrasound-induced air bubbles [13]. However, more research is needed to apply those techniques to PTT in clinics although it seems promising. To increase therapeutic depth and thus treatment efficacy, previously, we proposed the dual thermal therapeutic (DTT) method in which both light and US are transmitted into a treatment region at the same time [14]. The role of delivered US is to increase the temperature of the entire treatment area where the target chromophores exist, but the temperature is controlled below 40°C to avoid damaging normal cells from the US energy. Under this condition, the degree of temperature increase by the incident light can be lowered to induce coagulative necrosis of the target cells, as compared to the case of light irradiation only. This is so because the sum of the temperatures of the target cells increased by both US and light energies exceeds a threshold temperature at which coagulation occurs. As a result, treatment depth can be increased while retaining high target selectivity of PTT, which were verified through skin-mimicking phantom experiments. As another approach, it was proposed that light is used to treat a superficial area of the target lesion and the deeper lesion is treated using HIFU at the expense of selective therapeutic capability [15].

In this paper, we report the performance evaluation results of the DTT method using resected melanoma cancers and propose a pulse sequence for real-time DTT monitoring by photoacoustic (PA) imaging. The DTT method is based on the hypothesis that the temperatures generated by light and US energies, respectively, can be added constructively. Although we proved the validity of the hypothesis through the in-vitro experiments [14], the thermal addition effect needs to be confirmed in cancers and the ability of selective treatment should be assessed in real situations. Real-time treatment monitoring is also important for safe and highly efficacious DTT. Currently, the feasibility to use X-ray computed tomography (CT), magnetic resonance imaging (MRI), and PA imaging, and US imaging for image-guided PTT has been investigated [9,16–19]. Among them, US and PA imaging modalities are suitable for real-time treatment monitoring of PTT because they have real-time imaging capability [20]. For the DTT method, however, US imaging may suffer from high-intensity interference signals generated by therapeutic US although there are several ways to avoid the interference patterns at the cost of sacrificing frame rate or increasing computational complexity [21–23]. To this end, PA imaging is a good choice for real-time monitoring of DTT because the target chromophores can absorb light energy delivered for PA imaging as well as treatment. Additionally, since PA signal increases in proportional to the temperature of a medium [24,25], it is possible to identify the treatment area during DTT when monitoring the change in PA signal strength over time. Note that PA imaging also has the ability to visualize the heating area by high intensity focused ultrasound (HIFU) as well as laser [26,27]. One disadvantage of PA imaging is the inability to provide anatomical information except blood vessels. For this reason, combined PA and US imaging is the best solution for real-time treatment monitoring if possible. To take full advantage of PA imaging in DTT, therefore, the sequence of light and US transmission for treatment and imaging should be developed.
2. Materials and methods

Fig. 1. (a) Photograph of the melanoma-contained tissue-mimicking phantom and (b) illustration of experimental arrangement.

The ex-vivo experiments were conducted with the approval of the Institutional Animal Care and Use Committee at Sogang University. Female BALB/c mice (5-weeks old) were xenografted with the B16-F10 cell (5 × 10^6) into the right flank. After 4 weeks, melanoma cancer was extracted and embedded in the tissue mimicking phantom as shown in Fig. 1(a). The phantom consisted of the front section of 4 mm thickness and primary section containing the melanoma cancer. For the primary section construction, the mixture of a degassed water of 72% (v/v), an 40% (w/v) acrylamide of 17.3% (v/v), a TRIS buffer of 9.72% (v/v), a 10% (w/v) ammonium persulfate of 1.13% (v/v), and a tetramethylethylenediamine of 0.81% (v/v) were prepared. A bovine serum albumin (BSA) of 7.5% (w/v) was poured into the mixture while vigorously stirring, which was hardened at room temperature. To mimic normal tissue in which optical scattering and absorption occur, the front section phantom (see Fig. 1(a)) was constructed. The front section also served to provide evidence of whether transmitted US and laser energies induced coagulative necrosis before reaching the target region. For construction of the front section, an Evan blue of 0.01% (v/v) as optical absorbers and an intralipid-20% of 1% (v/v) as optical scatterers were added to the acrylamide-BSA based mixture. This mixed solution was poured onto the primary section. Indocyanine green (ICG, Dongindang Pharm. Cd. Ltd., South Korea) was used as a photothermal agent. Before placing the front section, 1 ml of ICG per gram of melanoma cancer was injected into the melanoma. In the ex-vivo experiments, the melanoma-contained phantom was immersed into a container filled with degassed deionized water.

To transmit US energy, a ring-shaped transducer (H102, Sonic Concepts, Bothell, WA, USA) with outer and inner diameters of 64 and 22.6 mm was used. The center frequency and geometric focal length of this transducer were 1.1 MHz and 62.6 mm. The electric pulses were generated using a function generator (AFG3252, Tektronix Corp., Beaverton, OR, USA) and amplified by 49 dB using a radio-frequency (RF) power amplifier (75A250 A, Amplifier Research Crop., Souderton, PA, USA). These pulses were applied to the transducer through a RF impedance matching network to minimize the loss of electrical power. The position of the transducer was adjusted using a motorized 3-axis XYZ stage (SGSP26-100, Sigma-KOKI Co., Japan) so that its focal area could be located 2 mm behind the cancer surface. The depth of the focal area was confirmed by inducing coagulation in the primary section of the melanoma-contained phantom, and the phantom was subsequently moved horizontally to position the melanoma cancer in the focal area. The amplitude, length, and repetition time of the electric pulse were determined so that transmitted US would cause the temperature in the focal region to be below 40°C. Several preliminary experiments were performed on the melanoma-contained phantoms to determine the maximum treatment time at which the
temperature of the focal area was below 40°C at a given spatial-peak pulse-average intensity (ISPPA); the maximum treatment time was found to be 40 s. Transmitted US with a pulse amplitude of 56.4 V, a pulse length of 60 ms, and a pulse repetition time of 100 ms yielded an ISPPA of 248 W/cm², and in this case the temperature of the focal region was changed from 30.7°C to 37.0°C after 40 s from the start of the energy transmission. When the pulse amplitude was increased to 70.5 V (i.e., ISPPA of 387 W/cm²), the temperature was raised from 30.2°C to 39.2°C, which was measured using a thermal camera (FLIR E60, Wilsonville, OR USA). For selective treatment, a continuous wave (CW) laser with a wavelength of 808 nm (MDL-III-808, CNILaser, China) was delivered into the target lesion through the central opening of the transducer (see Fig. 1(b)); the peak absorbance of ICG occurs at a wavelength of 800 nm [28]. Since the absorption rate of the 808-nm laser is very low in the melanin of melanoma [7], the effect of melanin concentration on the change of treatment depth could be avoided by using the CW laser. The non-focused laser beam had a Gaussian profile with a full-width at half-maximum (FWHM) of 3 mm after passing through a collimator (F280APC-B, Thorlabs, Inc., Newton, NJ, USA). The laser irradiation was controlled using a power and control module (PowerBox, RGB Photonics GmbH, Kelheim, Germany), i.e., irradiation on and off. The function generator was also used to synchronize the laser irradiation and the ultrasound transmission. When a peak laser power of 0.4 W was delivered for 60 ms, the local temperature of the ICG injection site was changed from 30.8°C to 36.4°C after the 40-s duration of therapy. The local temperature was 31.2°C to 45.5°C in the case of 1.64 W laser power. Note that this experiment was repeated three times using different melanoma-contained phantoms and the average local temperature was calculated.

Fig. 2. (a) Pulse sequence for real-time dual thermal therapy and (b) timing diagram of the pulse sequence used in this study.

To ascertain the feasibility of real-time treatment monitoring, the combined US and PA imaging probe, in which an US array transducer (L7-4, Verasonics Inc., Kirkland, WA, USA) was integrated with bifurcated optical fibers, was placed on top of the melanoma-contained phantom as shown in Fig. 1(b). A research US imaging system (Vantage Research Ultrasound System, Verasonics Inc.) was used for US imaging and PA signal reception. For PA imaging, a Nd:YAG laser excitation system (Surelite III-10 and Surelite OPO Plus, Continuum Inc., Santa Clara, CA, USA) was used to deliver laser with a length of 7 ns, a wavelength of 680 nm, and an energy density of 12 mJ/cm². The PA signals received by the US imaging system
were recorded and used to construct PA images with an adaptive beamformation algorithm [29,30] in MATLAB (MathWorks Inc., Natick, MA, USA).

Figure 2 shows the proposed pulse sequence for real-time DTT monitoring. Before DTT, an US image is acquired to confirm the location of the target lesion. At this stage, the focal area of the US therapeutic transducer can be visualized using the focal spot localization method [31], so that the transducer location can be adjusted to place its focal spot on the target lesion to ensure optimal treatment. After acquiring an initial PA image, DTT and subsequent PA imaging are performed repeatedly. In this study, DTT was conducted for 60 ms, and repeated every 100 ms (see Fig. 2(b)). Note that the minimum time required for PA imaging is approximately 6.5 μs when the maximum imaging depth is 10 mm and the sound speed is 1540 m/s.

3. Results and discussion

During DTT, the temperature of the melanoma-contained tissue-mimicking phantom was measured using the thermal camera as shown in Fig. 3. When both US with an I_{SPPA} of 248 W/cm² and laser with a peak power of 0.4 W were transmitted at the same time, the average temperature changed from 31.1°C to 46.3°C at 40 s. Note that the average temperature was obtained by averaging the maximum temperatures of each sample measured under the same conditions. Because individual delivery of each energy increased the average temperature from 30.7°C to 37.0°C in the case of the US energy and from 30.8°C to 36.4°C in the laser energy, this experimental result reaffirmed that the temperatures generated by light and US energies can be added constructively. Additionally, the result implies that the laser with a peak power of 0.4 W is incapable of inducing coagulative necrosis of the target lesion, but the US energy makes it possible to do so. The average temperature further increased from 31.7°C to 52.4°C when the laser power was increased to 1.64 W. Note that the maximum average temperature by the laser irradiation with a peak power of 1.64 W alone was 45.5°C. The temperature difference between the two cases was due to the transmitted US. When the US energy was increased to 387 W/cm², the maximum average temperature was also increased to 47.8°C in the case of the simultaneous delivery of the laser with a peak power of 0.4 W and 53.6°C with a peak power of 1.64 W (see Fig. 3(e) and (f)).

Fig. 3. Thermal images of the four representative samples of the ICG-injected melanoma, which were measured at 10 and 40 s from the start of DTT: (a) 248 W/cm² US and 0.4 W laser, (b) 248 W/cm² US and 1.64 W laser, (c) 387 W/cm² US and 0.4 W laser, and (d) 387 W/cm² US and 1.64 W laser. (e) and (f) show the temperature change as a function of the treatment time. The two dotted lines in (d) indicate the front section of the melanoma-contained tissue-mimicking phantom.
The US and PA images of the melanoma-contained phantom were acquired before DTT (Fig. 4(a) and (b)). Note that US with an ISPPA of 248 W/cm² and laser with a peak power of 0.4 W were used for the DTT. Since the optical absorbance of ICG is very low at a wavelength of 680 nm used for PA imaging, it can be considered that the PA signals in Fig. 4(b) were generated from the boundary between the phantom and melanoma and the melanin in the melanoma cells. When the 808-nm CW laser for DTT was transmitted into the target lesion, the injected ICG mainly absorbed the CW laser energy because ICG has the peak optical absorbance at 800 nm [28]. For this reason, the local temperature rise due to DTT occurred in the ICG injection region. During DTT, therefore, the strong PA signal appeared in the area indicated by the white dotted circle in Fig. 4(d) where no PA signals existed before DTT (see Fig. 4(b)). This implies that the target lesion did not contain a sufficient concentration of melanin to generate PA signals high enough to be detected, but the temperature rise by ICG absorbing the CW laser energy resulted in increasing the PA signals. In addition, this temperature rise led to coagulative necrosis in the target lesion, which was also confirmed by the US image acquired after DTT (Fig. 4(c)); the bright spot indicated by the red dotted circle was observed and its location matches well with that in the PA image. Note that the coagulation lesion appears bright in US images [4,21]. In addition, the coagulation lesion could be identified in the photograph of the cross-sectioned melanoma cancer: the dark area indicated by the red dotted circle in Fig. 4(e). In the PA images, it was observed that the signal strength from outside the focal region also increased during DTT. This is so because the transmitted US energy caused a local temperature rise outside the focal region, but this temperature rise did not contribute to the coagulative necrosis.
Figure 5 shows the combined US and PA images acquired at 0, 20, 40 s after transmitting US with an $I_{SPPA}$ of 387 W/cm² only (a), laser with a peak power of 1.64 W only (b), and both US and laser (c). The US images were acquired before each therapy and used for co-registration with the PA images. The location of the target lesions in the melanoma-contained phantom could be identified due to the US image that provided the information about where the PA signals were generated. From the visual assessment, it was seen that transmitting US alone did not lead to coagulative necrosis of the target lesion (see Fig. 5(a)). This was confirmed by measuring the PA signal strength in the US focal area indicated by the solid arrow and outside the focal area by the dashed arrow as shown in Fig. 6(a); the PA signal strength from both areas did not change considerably with the treatment time. This result also implies that the local temperature rise (from 30.2°C to 39.2°C) caused by the US energy was not enough to increase the PA signals. Note that the US image of the target lesion was 1.18 times brighter after therapy than before therapy (see Fig. 5(a)). On the other hand, the US image of the posterior portion of the cancer was 1.13 times brighter in the case of the before-therapy. This stemmed from a small change in the position of the phantom due to transmitted US pressure, rather than a change in acoustic properties of the tissue. In contrast, the laser
induced coagulative necrosis that appeared in the US image acquired after the treatment (see the area indicated by the solid arrow in Fig. 5(b)). However, the therapeutic depth was very low; the maximum coagulation depth was measured to be 1.48 mm. The PA signal strength increased 3.2 times in the target lesion, but this value was similar to the initial value outside the target lesion: only 0.34 times increase (see Fig. 6(b)). In the case of DTT, the coagulative necrosis occurred at a deeper depth that was 3.27 mm (see Fig. 5(c)), which implies that the proposed DTT is capable of increasing treatment depth by 2.2 times compared to the conventional PTT. The PA signal strength increased 5.8 times in the US focal area whereas the increase was 1.3 times out of focus. This increase in the PA signal strength in the US focal region was caused by the change in the optical property of the coagulation lesion [32] as well as the local temperature rise itself. Additionally, the increase rate of the PA signal strength outside the focal region was higher than that in the case of transmitting either US or laser only. This may result from scattering reduction due to the local temperature rise [33] and/or US-induced air bubbles [13] in the pathway of the incident laser (i.e., the US focal area), thus increasing the penetration depth of the laser. These causes of optical scattering reduction are other possible factors that lead to an increase in treatment depth in the proposed DTT because light is less attenuated and the light fluence contributing to the temperature rise of the target lesion increases. The reason that the PA signal was increased outside the US focal area will be investigated as a further work, which makes it possible for us to come up with one way to further increase therapeutic depth of DTT. PA imaging has the potential to quantitatively measure the local temperature of thermotherapy lesion by comparing the change in PA signal intensity during treatment with the initial value, and there have been several attempts to realize that capability [16,25,32]. However, the change in optical absorbance during thermotherapy hampers the accurate measurement of temperature, and the rate of this change may vary depending on the type of tissue. To use the potential of PA imaging for temperature measurement, therefore, intensive research into these issues should be undertaken for safe and highly efficacious DTT.

4. Conclusions
In this paper, it was demonstrated that the temperatures generated by light and US energies can be added constructively in the resected melanoma cancers, thus increasing the treatment depth while maintaining the selective treatment capability of PTT. We also showed that combined PA and US images are capable of providing the information about the location of treatment area in real time. This result indicates that the proposed pulse sequence works well for real-time monitoring of DTT. Since this study was conducted using ICG that is not the optimal photothermal agent, we believe that the therapeutic performances of DTT can be considerably improved if photothermal agents with high lesion targeting and high thermal conversion efficiency are used.

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Disclosures
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