In Vivo Ultrasound and Photoacoustic Image-Guided Photothermal Cancer Therapy Using Silica-Coated Gold Nanorods

Seungsoo Kim, Yun-Sheng Chen, Geoffrey P. Luke, and Stanislav Y. Emelianov

Abstract—In nanoparticle-augmented photothermal therapy, evaluating the delivery and spatial distribution of nanoparticles, followed by remote temperature mapping and monitoring, is essential to ensure the optimal therapeutic outcome. The utility of ultrasound and photoacoustic imaging to assist photothermal therapy has been previously demonstrated. Here, using a mouse xenograft tumor model, it is demonstrated in vivo that ultrasound-guided photoacoustic imaging can be used to plan the treatment and to guide the therapy. To evaluate nanoparticle delivery and spatial distribution, three-dimensional ultrasound and spectroscopic photoacoustic imaging of a mouse with a tumor was performed before and after intravenous injection of silica-coated gold nanorods. After injection and sufficient circulation of nanoparticles, photothermal therapy was performed for 5 min using an 808-nm continuous-wave laser. During the photothermal therapy, photoacoustic images were acquired continuously and used to measure the temperature changes within tissue. A heterogeneous distribution of temperature, which was spatially correlated with the measured distribution of nanoparticles, indicated that peak temperatures of 53°C were achieved in the tumor. An Arrhenius thermal damage model determined that this thermal deposition would result in significant cell death. The results of this study suggest that ultrasound and photoacoustic imaging can effectively guide photothermal therapy to achieve the desired thermal treatment.

I. INTRODUCTION

Photothermal therapy is a noninvasive cancer treatment method that utilizes the energy of light to produce heat energy that results in cancer cell death [1]. In nanoparticle-mediated photothermal therapy, near-infrared-absorbing nanoparticles (NPs) are introduced to tumors by intravenous injection. The nanoparticles circulate through the bloodstream and are delivered to the tumor site by several mechanisms, including the enhanced permeability and retention (EPR) effect and specific molecular targeting to the cancer cells based on cell proliferation or angiogenesis biomarkers [2]. After the nanoparticles have accumulated in the tumor, a continuous-wave (CW) laser (with its wavelength typically matched to the absorption peak of the injected nanoparticles) irradiates the tissue for localized thermal therapy. Continuous-wave lasers are used in photothermal therapy because the continuous deposition of energy enables faster, more uniform heating than a pulsed laser. Metallic nanoparticles, such as gold or silver, have been shown to effectively facilitate photothermal therapy performance because of their high optical absorption resulting from the surface plasmon resonance effect [3]–[9]. Furthermore, we have shown that coating gold nanoparticles with a thin layer of silica can even further enhance image-guided photothermal therapy performance because of increased thermal stability and photoacoustic signal amplification [10], [11].

To optimize therapeutic outcomes (i.e., to maximize thermal ablation of the cancerous tissue and to minimize damage to healthy tissue), the nanoparticle delivery and distribution must be confirmed before therapy and the temperature distribution must be monitored during therapy. Photoacoustic imaging is a versatile tool that can address both of these needs. First, spectroscopic photoacoustic imaging can be used to differentiate nanoparticles from biological tissue components such as oxygenated and deoxygenated hemoglobin [12]. This technique is based on the unique wavelength-dependent optical absorption properties of each tissue component. Because the photoacoustic signal amplitude can be correlated with the highly tissue-dependent optical absorption coefficient, it is possible to analyze the distribution of tissue components and nanoparticles. Therefore, spectroscopic photoacoustic imaging can be used for the confirmation of nanoparticle deposition in the tumor before therapy. Second, photoacoustic imaging can be used to estimate the temperature distribution during photothermal therapy [7], [13], [14]. Because photoacoustic pressure is a function of temperature, it is possible to correlate photoacoustic signal amplitude changes with temperature changes. This noninvasive and real-time temperature imaging method can provide enough information to estimate the thermal dose.

Photothermal therapy and photoacoustic imaging are complementary technologies in which nanoparticles act as either a therapeutic or an imaging agent. Although a CW laser is used in photothermal therapy, and photoacoustic imaging utilizes a nanosecond pulsed laser, both methods can use the same light delivery system. Absorption of the short laser pulse leads to a transient local pressure increase and the generation of a broadband pressure wave centered at the optical absorber, but the heating of the tissue is negligible. In turn, therapeutic CW laser irradiation does not significantly affect the photoacoustic signal generation, thus allowing photoacoustic imaging to
be used during the therapy. Overall, there is a synergy between photothermal therapy and photoacoustic imaging.

In this paper, we demonstrate ultrasound and photoacoustic image-guided photothermal therapy using an in vivo mouse model of cancer in which spectroscopic photoacoustic imaging was used to monitor nanoparticle delivery and photoacoustic-based thermal imaging was used to monitor the temperature during therapy.

II. Materials and Methods

A. In Vivo Mouse Model of Cancer

A 3-month-old immunodeficient nude mouse (Nu/Nu, Charles River) weighing 18 g was used for this study. Human epithelial cancer cells (A431 cell line) were subcutaneously injected on the right flank of the mouse to initiate tumor growth. The tumor grew for 20 d to a size of 10 mm in diameter. Prior to injection of nanoparticles (NPs), an imaging session was performed to acquire a baseline spectroscopic photoacoustic image of the endogenous tissue in the tumor region. A 200-μL silica-coated gold nanorod solution containing 400 μg of gold was then injected intravenously via the tail vein. Spectroscopic photoacoustic imaging of the tumor was repeated 63 h after nanoparticle injection. This time point has been shown to result in significant accumulation of nanorods in tumors with very few remaining in circulation [15]. During the imaging sessions, the mouse was anesthetized with a combination of isoflurane (0.5 to 2.0%) and oxygen (0.5 L/min). After the second imaging session, the mouse was euthanized by carbon dioxide asphyxiation and cervical dislocation. All procedures were performed following an animal protocol approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas at Austin.

B. Silica-Coated Gold Nanorods

Silica-coated gold nanorods were used in this study because of their enhanced thermal stability and photoacoustic signal response [16]. The silica-coated gold nanorods were produced from CTAB-stabilized gold nanorods [17] by exchanging CTAB with the biocompatible mPEG-thiol, and then using the mPEG polymer as a silane coupling agent for silica coating. The optical properties of gold nanorods were characterized by UV-visible (UV-Vis) extinction spectroscopy (Synergy HT, BioTek Instruments Inc., Winooski, VT). The shape and morphology of the gold nanorods are monitored by transmission electron microscopy (TEM) imaging using a Hitachi S-5500 field-emission scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a field-emission electron source operating at 30 kV. Fig. 1 shows the TEM image and the UV-Vis spectrum of the silica-coated gold nanorods used in this study. The average length and the width of the gold nanorods without silica coating were 39 ± 3 nm, and 9 ± 1 nm, respectively. After deposition of approximately 25 nm of silica coating, the average size of the rods grew to 63 ± 7 nm by 35 ± 5 nm. The UV-Vis spectrum indicates an optical absorption peak of silica-coated gold nanorods was centered at 805 nm. The silica layer caused a 20-nm redshift in the optical absorption peak.

C. Mouse Imaging Setup and Data Acquisition

The 3-D ultrasound (US) and photoacoustic (PA) imaging setup for the in vivo mouse experiment is shown in Fig. 2. An optical parametric oscillator (OPO) tunable laser (Spectra-Physics, Santa Clara, CA), producing a 5 ns laser pulse in the wavelength range of 400 to 2300 nm operating at 10 Hz was used as a radiation source. For this study, we used 8 wavelengths ranging from 740 to 840 nm in 20 nm increments. The average laser fluence at the surface of mouse skin was approximately 16 mJ/cm², satisfying the American National Standard Institute (ANSI) limit for human skin exposure [18] (~30 mJ/cm² for the wavelengths used in these studies). The pulsed laser was delivered to the tumor through an optical window attached to one side of a water tank. The water tank provided acoustic coupling between the array transducer (128 elements, 9 MHz center frequency, 5.8 MHz bandwidth, LA12/128, Vernon SA, Tours, France) and the mouse skin. Furthermore, the temperature in the water tank was held at a constant 37°C with a water circulation system (Isotemp 3013H, Fisher Scientific, Hampton, NH) during imaging to regulate the mouse body temperature. To keep both the transducer-cable connection and the head of the mouse above the water, the mouse bed was tilted at 45°. The bed was connected to a 1-D motorized system (National Instruments Corp., Austin, TX) to scan a 3-D volume of the mouse.
The array transducer was connected to either a Sonix RP ultrasound system (Ultrasonix Medical Corp., Richmond, BC, Canada) or a WP32 system (Winprobe Corp., North Palm Beach, FL) to collect either pulse–echo US or PA signals. The Sonix RP system was chosen for US imaging because of its ability to quickly capture high-quality US images with multiple focal zones, whereas the WP32 system allowed simultaneous access to 32 data channels needed for PA imaging. For one 2-D US image, 128 beams of RF signals (i.e., beamformed US data) were collected using 64 transmit and 32 receive channels, requiring an acquisition time of about 0.025 s. In PA imaging mode, 4 laser pulses were required for one full data set of 128 RF signals (i.e., pre-beamformed PA signals). Moreover, to enhance the SNR needed for reliable spectroscopic photoacoustic imaging and photoacoustic temperature mapping, 32 PA pre-beamformed RF signals were averaged, requiring 128 laser pulses for each PA image. The acquisition time for one frame of PA image was 12.8 s. The PA image reconstruction, spectroscopic analysis, and thermal imaging calculations were performed offline.

For photothermal therapy, an 808-nm, 1-W CW laser (Power Technology Inc., Little Rock, AR) was used to deliver light through a 600-μm-diameter optical fiber, irradiating the mouse skin and underlying tumor (Fig. 2). Ultrasound images were collected before and immediately following 5 min of CW laser irradiation. Photoacoustic signals were recorded during the CW laser irradiation.

\[ p_0 = \Gamma \mu_a F, \]  

(1a)

where \( \Gamma \) is Grüneisen parameter, \( \mu_a \) is the absorption coefficient of the medium, and \( F \) is the laser fluence. The Grüneisen parameter, \( \Gamma \),

\[ \Gamma = \frac{\beta v_s^2}{C_p}, \]  

(1b)

is defined by the volume expansion coefficient, \( \beta \), the speed of sound, \( v_s \), and the heat capacity at constant pressure, \( C_p \).

At a constant temperature, the Grüneisen parameter at the same position (i.e., same type of tissue) can be assumed as a constant value. In addition, compensating for the fluence at different wavelengths [18], multi-wavelength photoacoustic pressures can be correlated with the optical absorption coefficient of the various types of tissues and the injected nanoparticles.

Because there are three major components (i.e., oxygenated hemoglobin, deoxygenated hemoglobin, and nanoparticles) that dominantly absorb the light energy (i.e., produce photoacoustic signal) in the wavelength range of 740 to 840 nm, the following spectrum was compared with the photoacoustic spectrum measured within each 500 μm³ voxel:

\[ \mu_{\text{HbO}_2} + \mu_{\text{Hb}} + \mu_{\text{NP}} = \mu, \]  

(2)

where \( \mu_{\text{HbO}_2}, \mu_{\text{Hb}}, \) and \( \mu_{\text{NP}} \) are the normalized absorption spectra of oxygenated and deoxygenated hemoglobin, and nanoparticles, respectively. \( C_{\text{HbO}_2}, C_{\text{Hb}}, \) and \( C_{\text{NP}} \) are the relative concentrations of oxygenated and deoxygenated hemoglobin, and nanoparticles within the volume, respectively. The total relative concentration was set to 1 (i.e., \( C_{\text{HbO}_2} + C_{\text{Hb}} + C_{\text{NP}} = 1 \)). The concentrations \( C_{\text{HbO}_2}, C_{\text{Hb}}, \) and \( C_{\text{NP}} \) were determined numerically by minimizing the mean square error of (2) [19].

\[ E. \text{Photoacoustic-Based Thermal Imaging} \]

Photoacoustic imaging can be used to monitor the temperature distribution in tissues noninvasively [11], [13]. Photoacoustic signal intensity is directly proportional to the dimensionless Grüneisen parameter [see (1a)]. The volume expansion coefficient and the speed of sound [see (1b)] are both temperature-dependent and linearly proportional to the temperature of water-based and fatty tissues between 10°C and 55°C [7], [20], [21]. Therefore, according to (1), the temperature change can be obtained by monitoring the photoacoustic signal amplitude.

Generally, tissues are weak optical absorbers, requiring plasmonic nanoparticles for efficient photothermal therapy [7]. Similarly, tissues produce weak photoacoustic signals compared with nanoparticles. Therefore, the benefit of nanoparticles is twofold: they enhance the photoacoustic contrast and facilitate the photothermal therapy. However, in the case of nanoparticles, the stress and the
where $\sigma$ and $N$ are the absorption cross-section and number of the absorbing nanoparticles, respectively, and $\eta$ is the heat transfer efficiency. The effective Grüneisen parameter $\Gamma_{\text{eff}}$ is that of the signal-generating water, and depends on the thermal expansion coefficient of water. Nevertheless, similar to (1), the relation between photoacoustic signal and temperature remains linear for the nanoparticle suspension. We experimentally confirmed the linear dependence of the photoacoustic amplitude [22], and suggested that silica-coated gold nanoparticles can produce higher photoacoustic signals because of their higher heat-transfer efficiency [10]. Therefore, for water-based tissue and suspension of hydrophilic nanoparticles in water, the photoacoustic signal is linearly proportional to temperature

$$p_0 = \eta \Gamma_{\text{eff}} N \sigma F,$$  \hfill (3)

where $\alpha$ is a parameter that can be estimated by making photoacoustic measurement at baseline temperature (i.e., 37°C) and using the fact that the photoacoustic signal at 4°C is zero [22], [23].

It is important to note that the volume expansion coefficient of tissue dominates this dependence on temperature; changes of speed in sound are minimal for the 10°C to 20°C temperature increases in photothermal therapy [7]. Because of the relatively small changes in the speed of sound, the irradiation of the tissue with a CW laser during photoacoustic imaging does not impact the stress confinement condition.

In principle, the spatial resolution of thermal imaging is limited only by the resolution of the imaging system. In this case, the resolution is limited by the ultrasound transducer to approximately 250 to 300 μm. To account for respiratory motion, we applied a low-pass spatial filter before thermal processing. This resulted in a spatial resolution of approximately 750 μm.

An Arrhenius thermal damage model was used to estimate cell death in the tumor from the measured temperature distribution [24]. The thermal dose was calculated in units of cumulative effective minutes at 43°C (CEM43) by the following equation:

$$\text{CEM}43 = \sum_{T = \text{final}}^{\text{final}} R^{43 - T} \Delta t,$$  \hfill (5)

where $T$ is the measured temperature and $\Delta t$ is the imaging interval. Furthermore, in (5), $R = 0.25$ if $T \leq 43$, and $R = 0.5$ if $T \geq 43$. It is typically assumed that CEM43 = 60 min corresponds to complete cell death in a region.

### III. RESULTS AND DISCUSSION

#### A. Monitoring of Nanoparticle Delivery Before Therapy

The mouse was imaged at two different time points: before the injection of nanoparticles—which acted as a control and 63 h after the injection of silica-coated gold nanorods. The 3-D ultrasound image in Fig. 3 shows anatomical features of the tumor (i.e., location and size). The photoacoustic images obtained before nanoparticle injection show the intrinsic optical absorption contrast, which is mainly produced by blood.

The photoacoustic images obtained after administrating nanoparticles include signals from not only blood, but also nanoparticles. A single-wavelength photoacoustic image cannot differentiate nanoparticles from blood. However, the results of spectroscopic photoacoustic imaging clearly show spectrally unmixed oxygenated blood, deoxygenated blood, and nanoparticles. All three components were displayed from 50% to 100% of relative concentration.

Spectroscopic photoacoustic imaging shows no nanoparticle deposition in the tumor before the injection but clearly shows the presence of the nanoparticles after the injection. These results suggest that spectroscopic photoacoustic imaging can be reliably used for monitoring nanoparticle delivery.

#### B. Monitoring of Temperature Rise During Photothermal Therapy

Once the nanoparticles were injected and allowed to circulate for 63 h, photothermal therapy was performed for 5 min using a CW laser wavelength of 808 nm. During therapy, photoacoustic images from a single plane were collected at 30-s time intervals using a pulsed laser operating at 800 nm wavelength. Fig. 4 shows the ultrasound/photoacoustic images obtained before CW laser irradiation, and at 1 and 3 min after the CW laser was turned on. The arrows in Fig. 4 indicate the location and direction of CW laser irradiation. The CW laser beam spot size on the skin of mouse was 0.8 mm in diameter.

Based on (4), a temperature distribution was estimated throughout the tissue. A maximum temperature rise of 16°C during therapy was observed by photoacoustic-based thermal imaging. During the first minute, the maximum temperature in the mouse tumor quickly increased by 10°C, and then the temperature gradually increased up to 16°C above body temperature until the CW laser was turned off (at 5 min). Because the mouse was placed in a temperature-controlled water tank, it was assumed that the initial temperature in the tumor was 37°C.

During therapy, the temperature increased to as high as 53°C. The temperature profile in the tumor is nonuni-
form, suggesting an uneven distribution of optical absorbers and, therefore, temperature deposition. Furthermore, blood circulation and heat diffusion add to temperature variations. This means that the tissue response to photothermal therapy will also be inhomogeneous; some regions will experience more cell death than others. In the areas with the greatest amount of heat, the Arrhenius thermal damage model, shown in (5), predicts significant cell death. In fact, the temperature profile shown in Fig. 4(g) results in maximum temperature rise of about 16°C in the tumor.

It is important to note that the regions of increasing temperature are well correlated with the regions of nanoparticle accumulation (Fig. 5). This result suggests that it is nanoparticles that are acting as the heat sources, not the tissue. Therefore, nanoparticles are critical for efficient and effective photothermal therapy.

Fig. 3. (a)–(e) 3-D ultrasound, photoacoustic, and spectroscopic photoacoustic images of before nanoparticle injection, and (f)–(j) after the injection. [(a) and (f)] Ultrasound images show tumor size and location. [(b) and (g)] Photoacoustic images were obtained at wavelength of 800 nm. Spectroscopic photoacoustic images represent relative concentrations (50% to 100%) of [(c) and (h)] oxygenated hemoglobin, [(d) and (i)] deoxygenated hemoglobin, and [(e) and (j)] nanoparticles. The field of view for each volume is 22.4 mm (width) by 25.8 mm (depth) by 20.0 mm (length).

Fig. 4. (a)–(c) Combined ultrasound and photoacoustic images, and (d)–(f) photoacoustic-based thermal images. The arrow represents the direction of CW laser irradiation (808 nm, 1 W). Photothermal therapy of 5 min results in maximum temperature rise of about 16°C in the tumor.
Fig. 5. (a) Nanoparticle distribution obtained from spectroscopic photoacoustic imaging and (b) temperature distribution after 3 min of photothermal therapy obtained from photoacoustic-based thermal imaging. Nanoparticle distribution and temperature distribution are closely related to each other.

Although the ultrasound imaging was performed in real time, the photoacoustic images were obtained at a slow rate because of limitations of the imaging system, including the limited number of channels and finite electronic signal-to-noise ratio. Because of these limitations, significant averaging was performed, resulting in slow temporal resolution of the system. Nevertheless, it was sufficient to demonstrate the ability of ultrasound/photoacoustic imaging to monitor the temperature increase during photothermal therapy.

In the current studies, the silica-coated nanorods accumulated in the tumor because of the enhanced permeability and retention (EPR) effect associated with leaky vasculature. However, nanoparticles can be bioconjugated to increase the concentration of nanoparticles at the tumor [25]. For example, the epidermal growth factor receptor (EGFR), which is overexpressed in many cancers, can be targeted using antibodies.

Physiological (e.g., cardiac or respiratory) motion could introduce artifacts in the spectroscopic photoacoustic imaging and photoacoustic temperature monitoring. However, given that photoacoustic temperature imaging relies on the measurements of signal strength, it is not as sensitive to tissue motion as, for example, ultrasound thermal imaging [7]. Furthermore, ultrasound motion tracking could be used to reregister the photoacoustic images to the initial grid. Finally, it is possible to use an electrocardiogram (ECG) to trigger data capture and collect data frames at the same point in the cardiac cycle to potentially reduce motion artifacts [26].

IV. Conclusion

We have shown that ultrasound and photoacoustic imaging techniques can be used for planning and guiding photothermal cancer therapy. Ultrasound imaging provides anatomical information (i.e., spatial location, margins, and size of the tumor). Spectroscopic photoacoustic imaging gives functional information, such as hemoglobin content and nanoparticle distributions. Photoacoustic-based thermal imaging offers temperature distribution during photothermal therapy. All of these imaging techniques were developed and optimized for, and demonstrated on, an in vivo mouse model of cancer. Silica-coated gold nanorods were used because of their thermal stability and enhanced photoacoustic response.

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References


