Internal temperature increase during photothermal tumour ablation in mice using gold nanorods

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***Abstract*— Laser ablation (LA) is gaining large acceptance in the treatment of tumor. One of the main risks of this treatment is damaging the healthy tissue around the tumor. Among the solutions proposed to improve the selectivity of the LA and to localize heating to tumor tissue, the use of gold nanoparticles is one of the most promising.**

The aim of this work is threefold: *i)* to measure the temperature increase within the tumor during plasmonic photothermal therapy using gold nanorods; *ii)* to investigate the influence of nanorods concentration and laser settings on both the intra-tumoral temperature and the tumor surface temperature; *iii)* and to establish the nanorods concentrations able to cause tumor resorption at a defined laser settings.

Two sets of trials were performed: *i)* 16 mice were divided in four groups with different treatment time (i.e., 5 min, 2min, 1min, and 30s), with constant gold nanorods amount (i.e., 12.5µg) and laser power (i.e., 3W·cm^{-2}); *ii)* 16 mice were divided in four groups treated with different amount of gold nanorods (i.e., control, 12.5µg, 25µg, 50µg) for 5 min at 2W·cm^{-2}. Results show significant differences between internal and surface temperatures. We also demonstrate that this temperature difference increases with nanoparticle concentrations, decreases with laser power, and is not impacted by treatment time. This information is critical to improve the theoretical models that will guide future study designs in sensitive orthotopic tumor models.

I. Introduction

Thermal ablative techniques –i.e., radio-frequency, microwave, laser ablation (LA), high intensity focused ultrasound (HIFU)– are gaining acceptance as a valid alternative to traditional surgery for the treatment of many focal malignancies, and for patients who are not good surgical candidates [1]. They are less invasive, less painful and permit faster recovery times compared to traditional surgery.

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The goal of these therapies is to induce coagulative necrosis by elevating tumor temperatures, while limiting thermal exposure to surrounding tissue. LA is particularly attractive since it is amenable to MR-guidance of small, flexible fiber optics to reach targets in deep-lying organs [2,3]. Since its introduction in 1961 [4], LA has been used clinically as early as the 1980's for treating brain, gastrointestinal, and prostate tumors [5,6]. Much is known about the temperatures achievable with LA in both tumor and off target tissues. Unfortunately, it has proven extremely challenging to sufficiently heat tumor margins without causing significant damage to surrounding healthy tissue given their similar light absorption coefficients. Incomplete ablation and disease recurrence is common.

To overcome this hurdle, plasmonic photothermal therapy (PPT) is being developed to enable tumor-specific light absorption and tumor-localized heating. PPT utilizes biologically inert gold nanoparticles (AuNPs) to convert near infrared (NIR) light into thermal energy. While selectively deposited AuNPs should amplify heat within the tumor, it is not yet clear if this approach can be used to avoid significant collateral damage. Most animal studies to date have been performed using subcutaneous tumors in which off-target tissue damage affects non-essential skin or muscle within the flank. Few attempts to treat orthotopic tumors involving critical organs have been reported, because obvious burns to off-target tissue is usually observed. Before treatment of orthotopic tumors is feasible, an increased understanding is needed about the heat map generated by light-activated AuNPs.

Several theoretical models have predicted how AuNPs will impact heat conduction within tumor tissues exposed to NIR light [7]. Most do not consider variations in AuNP concentration, laser power (P), treatment duration (t), or anatomical tissue and blood perfusion differences. Furthermore, the predictions have not been validated experimentally [7], or utilize *ex vivo* tissue phantoms which poorly mimic heat transport in live tissue [8]. The few studies which monitor temperatures *in vivo*, use thermographic cameras that are limited to tumor surface measurements [9]. Thus, there is a critical need for information about intra-tumoral temperatures during PPT.

Here we utilize thermocouples to measure real-time intra-tumoral temperature (T_{IT}) during PPT. We demonstrate that T_{IT} is significantly higher than those measured on tumor surfaces (T_S) by either thermographic cameras or surface temperature probes. We further begin to establish how the T_{IT} changes as a function of P, t, or AuNP concentration. Moreover, we measure for the first time, the T_{IT} induced within tumors undergoing PPT

settings that are known to cause resorption of small breast tumors in mice [10]. This study provides the foundation from which more accurate theoretical models can be built; and will accelerate safe and effective transfer of PPT technology to relevant orthotopic tumor models.

II. Materials and Methods

Breast Xenograft establishment and AuNP injections:

Firefly luciferase expressing MDA-MB-231 human breast cancer cells were injected into the flank of nude mice (1e6/mouse). Seven days later, xenogen imaging was performed to confirm viable engraftment, then mice were divided into treatment groups (3-4/grp) before receiving intratumoral injections of either saline or select AuNP quantities (Nanopartz, peak absorption 808nm).

Photothermal ablation:

Three days post-intratumoral injection, photothermal ablation was performed. Mice were anesthesized with isoflorane (1.5 L/oxygen, 4%isoflurane). To protect the mice eyes, optical lubricant was applied and then shielded from the laser using black felt. Glycerol was applied to the tumor, then it was exposed to continuous wave 810nm light for up to 5 min (RPMC Lasers, Inc., O'Fallon, MO, USA). P was set to either 4.5 W or 3 W, which corresponds to a power density, PD, of 3 $W \cdot cm^{-2}$ or 2 $W \cdot cm^{-2}$.

Temperature monitoring:

During the procedure both the T_{IT} and the T_S were measured by two K-type thermocouples, connected to a data acquisition system (FX100, Yokogawa) with a sample period of 2 s. The first thermocouple was inserted into the tumor; the second one was placed on the mouse skin adjacent to the tumor. The position of the two thermocouples during the irradiation is shown in Fig. 1. In addition, an IR thermographic camera (FLIR Systems, A655sc) was used to measure peak sample surface temperature.

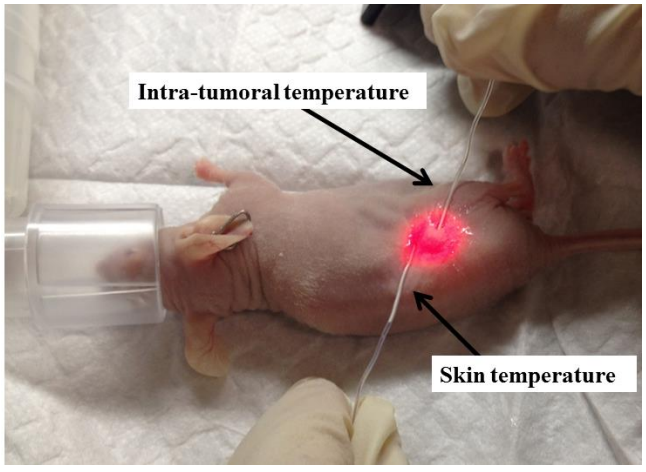

Figure 1. Experimental setup during the photothermal ablation: the thermocouples used to measure the skin temperature and the intra-tumoral one are shown.

Tumor monitoring:

Tumor resorption was monitored two days after laser exposure via Xenogen imaging. Mice were anesthetized with isoflorane, then received an intraperitoneal injection of D-luciferin suspended in PBS at 4.29 mg/mouse. Light emission was measured 9 min after injection of luciferin over an integration time of 10 s using a charge-coupled device camera (Xenogen IVIS-100). Living Image software was used to analyze resultant tumor flux. All procedures were approved by the City of Hope Institutional Animal Care & Use Committee.

III. Results

Observed difference between TIT and TS during ablation

Experiments were initiated using laser settings previously confirmed to be tumor ablative (continuous wave 810nm light, 5 min, 3 $W \cdot cm^{-2}$) [10]. At these settings, the temperature of any tissue exposed to the 1.5 cm spot size begins to steadily increase even in the absence of AuNPs (Fig. 2A). It reaches ~85% of its maximum temperature increase (12.8 ± 0.1˚C) within the first 2 min, then plateaus. Interestingly, the addition of 12.5µg AuNR inside the tumor neither increases the overall surface area of heated tissue, nor prolongs the time over which temperature increases. A higher overall temperature is achieved on the surface of AuNR injected tumors. The AuNP-associated temperature increase remains localized near the AuNP injection site, dissipating before it reaches the 1.5 cm boundary warmed the laser (Fig. 2A).

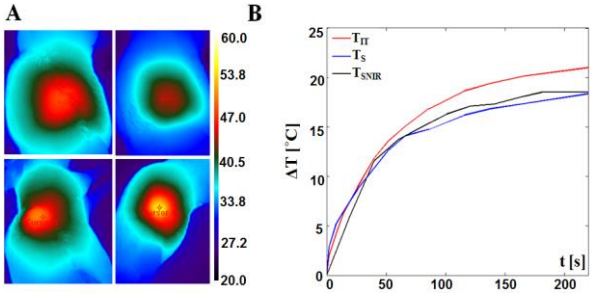

Figure 2. Temperatures recorded during photothermal ablation. A) Thermographs recorded in xenografts pre-treated with either saline (top panel) or 12.5 ug AuNR (bottom panel). B) Average temperatures measured over time using thermographic camera (black line), or thermocouples located either on the skin surface (blue line) or intra-tumorally (red line).

Our data demonstrate a very tight correlation between T_S measured via the thermographic camera relative to those measured using the thermocouple positioned on the skin surface (Fig. 2B). However, the T_S did not match those present within the tumor, which were on average 5˚C higher for the AuNP treated tumors. The rate of temperature increase measured in AuNP-loaded tumors was similar to that observed in the control tumors, reaching ~90 % of its maximum temperature (18.5 ± 3˚C) within the first 2 min before reaching a plateau (Fig. 2B). These data suggest that temperature increases of ~23 ± 3˚C are being achieved within tumors that are later resorbed, whereas tumors that only increase ~17 ˚C internally are not resorbed. Assuming that the initial temperature was the systemic

temperature of the mouse, this datum is consistent with temperatures required by traditional LA in that temperatures need to be elevated >55°C-60°C for a few minutes to destroy the tumor.

Maximizing difference between T_{IT} and T_S

We found that mice receiving saline injections experienced neither skin burns, nor tumor resorption. In contrast mice receiving 12.5 µg AuNP injections experienced both tumor resorption and burns. The burns wound healed spontaneously within a couple of weeks. Because off-target burning of the skin is always observed under these laser settings, our next goal is to adjust our t, P, and AuNP concentration to achieve tumor resorption, while minimizing the T_S.

Trial 1: Decreasing Laser treatment Duration

Initial efforts involved keeping the P value and AuNP concentration constant, but decreasing t. Decreasing the amount of time tissues were exposed to the laser should decrease the overall temperatures experienced by both the tumor and the skin surface, as shown in Fig. 3B. We found that mice receiving saline injections experienced neither skin burns, nor tumor resorption. In contrast, mice receiving 12.5 µg AuNP injections experienced both tumor resorption and burns (Fig. 3A). While burns were avoided in tumors exposed to laser for just 30 s or 1 min, the tumor was not resorbed 2 days after PTT. Furthermore, burn was observed in one of the mice exposed to the laser for 2 min (consistent with the T_{IT} reaching almost 60°C). Even in this case, the tumor was not resorbed as assessed via xenogen imaging.

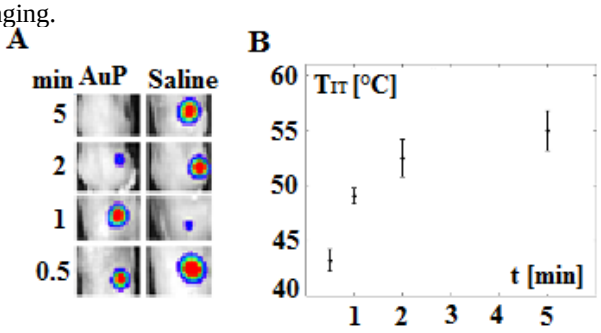

Figure 3. Efficacy and internal tumor temperature as a function of PTT duration. A) Xenogen images showing ffluc positive tumor cells 2 days after 3 W·cm^{-2}. PTT was applied for decreasing durations. B) Mean value of the intra-tumoral temperature recorded in each tumor after exposed to laser for increasing durations.

Trial 2: Decreasing Laser Power & Increasing [AuNP]

Our next efforts involved trying to increase the differential between the temperature plateaus that occur after 2 min of laser exposure. It should be possible to decrease the heat energy generated from off-target tissue absorption by decreasing P of the NIR light. While this would presumably decrease the AuNP absorption as well, perhaps the loss could be compensated simply by increasing the dose of AuNPs within the tumor. Thus, we exposed tumors for 5 min with a PD of only 2 W·cm^{-2}, but that were injected with either saline, 12.5µg, 25µg, or 50µg of AuNP per tumor. Table I shows the laser settings employed for each group.

TABLE I. LASER SETTINGS AND AMOUNT OF NANORODS INJECTED

	N	*P [W]*	*PD [W·cm^{-2}]*	*λ [nm]*	*Nanorods [µg]*
Group 1	4	3	2	810	12.5
Group 2	4	3	2	810	25
Group 3	4	3	2	810	50
Group 4	4	3	2	810	0

Our results from this trial show that decreasing the PD from 3 to 2 W·cm^{-2} did not significantly reduce the maximum T_{IT} recorded inside AuNP-free tumors, which still reached about 45 °C (Fig. 4B). While no skin burns were observed in the control mice, the mice that received AuNP injections of 12.5µg, 25µg, or 50µg of AuNP all experienced skin burns. While increasing the AuNP concentration within the tumor increased T_{IT}, no mice reached internal temperatures of 60°C. In all mice that received AuNP injections except those in group1, tumor resorption was also observed. The heating kinetics in each treatment group were similar, achieving ~85% of the maximum temperature in 2 min.

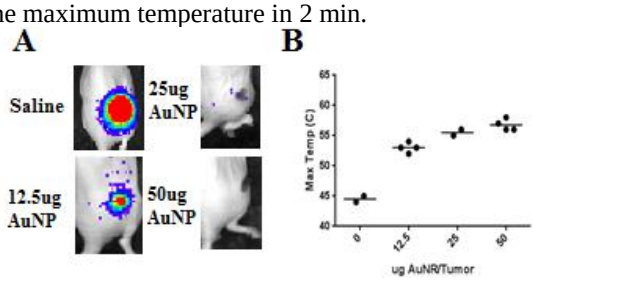

Figure 4. Efficacy and internal tumor temperature as a AuNP concentration. A) Xenogen images showing ffluc positive tumor cells 2 days after 2 W·cm^{-2} PTT was applied to tumors injected with increasing AuNP doses. B) Maximum internal temperatures recorded in each tumor using thermocouple.

The heating efficiency of the PTT is expressed as:

$$eff = \frac{\Delta T_{IT\_AuNR}}{\Delta T_{IT\_control}} \qquad (1)$$

where ΔT_{IT_AuNR} is the maximum T_{IT} increase experienced by the tumor during the photothermal ablation in the group with nanoparticles, and $\Delta T_{IT\_control}$ is the one experienced by the control group [11, 12,13]. Our data suggest that the heating efficiency increases with the AuNR concentration: it was 1.69, 1.84 and 1.99, for the group 1 (12.5µgAuNR/tumor), 2 (25µgAuNR/tumor) and 3 (50µgAuNR/tumor), respectively.

IV. DISCUSSION AND CONCLUSION

It is well known that using optimal laser settings during LA are important, however there still are very few *in vivo* studies specifying how alterations in laser parameters influence tumor resorption [14,15]. In the present study, we show that skin surface measurements provide insufficient information

regarding the intra-tumoral temperature increase that occur during PPT. The intra-tumoral temperatures are on average 5°C higher than those observed on the skin surface. The results observed here are consistent with two other studies (Hirsch *et al.* [12] and Dickerson *et al.* [13]), that also performed intra-tumoral thermometry.

The increase of peak T_{IT} and of the efficiency with the AuNR concentration highlights the efficacy of PPT when exposed at NIR light. The values of efficiency obtained in this study are lower than the one obtained in [12] and [13] for direct injection of nanorods (>3) and similar to the one obtained by in [13] for intravenous injection of pegylated gold nanorods (≈1.9). This difference can be motivated by the fact that they used different laser settings, different nanoparticles and concentrations with respect to our experiments.

We expand upon these previous studies by further demonstrating how the T_{IT} is influenced by P or PD, t, and AuNR concentration. Albeit preliminary, these results suggest that tumor resorption requires the T_{IT} exceed 50 °C for more than 3-4 min. On the other hand, in the groups in which the tumor did not resorb, the peak values of TIT are lower than in the group 1 or not sustained at high temperatures for longer than 2 min. In the AuNP-free control group, the T_{IT} is always lower than 45 °C. These results provide useful information for optimizing the amount of AuNR injected to improve procedural outcomes.

The results from trial two suggest the T_{IT} required for ablation could be achieved while reducing the off-target tissue damage if even lower P (or PD) were explored in conjunction with higher AuNP concentrations. In the future the analysis of the influencing factors on the effect of PPT in terms of its efficiency, T_{IT}, and tumor damage will be further investigated to take into account both the influence of AuNR concentration and the influence of laser settings. However, in order to rationally select the most promising P and corresponding AuNP concentration, finite element modeling should be employed. Given the multitude of experimental combinations that need to be tested to tease out the appropriate way to manipulate P, AuNP concentration, and t, a high-throughput, predictive approach must be employed.

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