Evaluation of IR780 and Rose Bengal as Sonothermal Agents

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***Abstract*— Sonothermal therapy (STT) is a targeted modality of cancer treatment that increases the temperature locally by utilizing thermal sensitizers and ultrasound. A major challenge of STT is providing effective hyperthermia which is above 50°C to the target area without the risk of damaging healthy tissues. This study investigates IR780 and Rose Bengal (RB) as potential sonothermal sensitizers. IR780, known for its effectiveness in photodynamic and sonodynamic therapy, has shown promise in generating reactive oxygen species, but its sonothermal efficacy remains unexplored. Similarly, RB, as a water-soluble xanthene dye has potential as a sonosensitizer, yet its efficacy in STT performance is not well-studied. This research investigates the temperature increase of culture media with or without incubation with these agents under ultrasound application at different powers to investigate the STT efficacy of IR780 and RB.**

Keywords*— *ultrasound hyperthermia; IR780; Rose Bengal; sonothermal therapy

I. Introduction

Sonothermal therapy (STT), which combines ultrasound with thermal sensitizers, has the potential to improve cancer treatment [1]. Traditional hyperthermia depends on only externally applied heat while sonothermal therapy uses the sensitizers that lead to localized temperature increases when exposed to ultrasound [2]. The main advantage is the ability of these sensitizers to convert the mechanical energy from ultrasound into thermal energy, allowing for more precise and targeted heating of the target area compared to conventional methods [3-6]. Moreover, ultrasonic waves, which are mechanical waves with frequencies above the range of human hearing, can penetrate tissue up to several centimeters deep while causing minimal or no harm to surrounding healthy tissues [1].

There are two main types of sonothermal therapy (STT): hyperthermal STT, which involves temperatures above 50°C to kill cancer cells but may also harm healthy tissues, and mild STT, which maintains temperatures between 42 and 50°C to minimize side effects but may be less effective due to the possibility of cancer cells developing resistance to heat [7]. Therefore, identifying sensitizers capable of achieving the optimal balance between effective thermal ablation and minimal side effects is critical.

Among the sensitizers explored for therapeutic purposes, IR780 and Rose Bengal have shown promise in related fields such as photodynamic and sonodynamic therapy. However, their role as sonothermal agents has not been as extensively studied. IR780 is a near-infrared (NIR) dye known for its effectiveness in deep-tissue targeting due to its hydrophobic nature and strong NIR absorption [3, 8, 9]. On the other hand, Rose Bengal, a water-soluble xanthene dye, has been explored for its sonodynamic effects, particularly in generating ROS in cancer cells. Both agents are known for their ability to generate reactive oxygen species (ROS) when irradiated by light or ultrasound, making them potential candidates for sonothermal applications [10-13]. However, despite their established roles as ROS generators, their sonothermal properties remain largely unexplored.

Previous studies have largely focused on the sonodynamic effects of these agents rather than their thermal contributions. For instance, Li et al. investigated the application of IR780 in sonodynamic therapy, using a concentration of 800 µg/mL and 2 W/cm² ultrasound on 4T1 mammary carcinoma in BALB/C mice [14]. Their findings showed no significant increase in tumor temperature during treatment, as measured by an electronic thermometer. These results indicate that IR780, while effective as a sonosensitizer for ROS generation, may have limited efficacy in direct temperature elevation, which is crucial for STT applications.

On the other hand, Kim et al. examined the use of a Rose Bengal derivative (RB2) in combination with pulsed high-intensity focused ultrasound (HIFU) on MDA-MB-231 breast cancer cells [15]. In vitro studies demonstrated that pulsed HIFU, with a peak negative pressure of >7 MPa (~27 W acoustic power), destroyed over 95% of breast cancer cells in suspension with >10 µM of RB2. However, temperature tests revealed that the treatment led to only a 10°C rise in temperature, which did not significantly impact cell viability. In their in vivo studies, even at high ultrasound powers (39 W peak acoustic power) and a dose of 25 mg/kg of RB2, the temperature rise in the tumors was less than 5°C. The limited thermal outcomes from these studies suggest that while IR780 and Rose

Bengal exhibit promise as sonosensitizers, their ability to generate sufficient temperature increases under ultrasound has not been fully established.

Given the advantages of IR780 in deep-tissue targeting and Rose Bengal's potential in superficial or vascularized tumors, this study aims to systematically compare their sonothermal effects under varying ultrasound powers. By prioritizing temperature elevation as the key metric, we aim to determine how effectively these agents function as thermal sensitizers in STT, moving beyond prior studies that focused mainly on cell viability and ROS generation. Through this investigation, we aim to provide critical insights into the feasibility of using IR780 and Rose Bengal in sonothermal therapy.

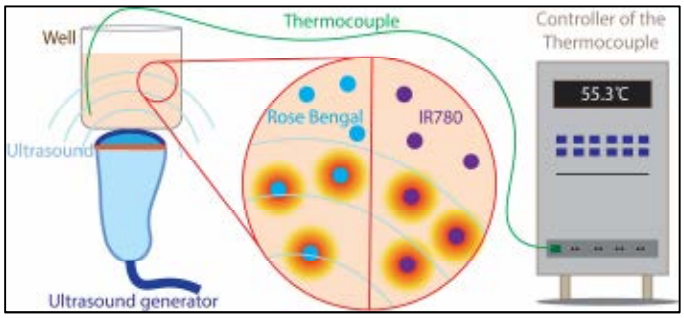


Fig. 1. Illustration of the experiment setup

II. Materials and Methods

A. Materials

IR-780 iodide, RB, and dimethylsulfoxide (DMSO, 99.9%) were purchased from Sigma-Aldrich. Cell culture reagents used were RPMI 1640, fetal bovine serum (FBS), and penicillin/streptomycin solution purchased from Capricorn Scientific.

B. Experimental Setup

The measurement set-up included a therapeutic ultrasound unit (Sonoplus 190, Enraf-Nonius B.V., Rotterdam, Netherlands) with a 5 cm^2 ultrasound head. Ultrasound head was covered with contact-gel. The temperature was measured by BAT-10 multipurpose thermometer from PhysiTemp, Inc., equipped with T-type thermocouples. The measurement set-up is shown in Fig.2.

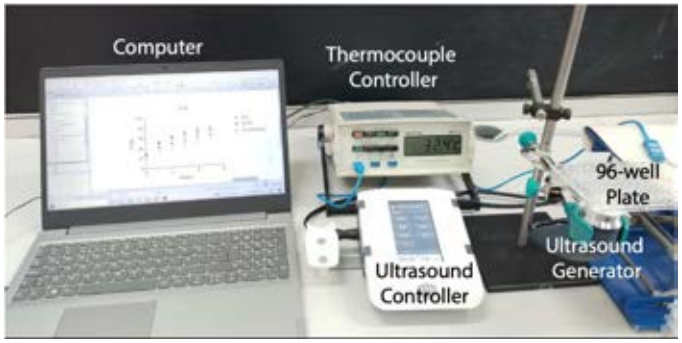


Fig. 2. Experimental setup.

C. Temperature Measurements

To evaluate the sonothermal performance, 100 µL of 5 µg/mL IR780 and RB were dispersed in culture medium (supplemented with 10% FBS and 5% penicillin and streptomycin) and added in a 96 well-plate to mimic the in-vitro conditions. Then 5 min ultrasound was applied at different intensities (1-6 W, 1 MHz, 50% duty cycle) and temperature was recorded each minute. The experimental plan parameters can be seen in Table I. All experiments were performed in triplicate.

TABLE I. EXPERIMENTAL GROUPS

Sample	*Ultrasound Power (W)*	*Duration (min)*	*Concentration (µg/mL)*
IR780 in Culture Medium	1, 2, 3, 4, 5, 6	5	5
RB in Culture Medium	1, 2, 3, 4, 5, 6	5	5
Culture Medium	1, 2, 3, 4, ***5***, 6	5	N/A

Summary of experimental groups.

III. Results

Figure 3 shows the temperature changes in culture media with without the potential sonothermal agents, IR780 and Rose Bengal (RB), after applying various ultrasound power levels. The data indicate that ultrasound application led to a temperature increase in all experimental groups, with or without sensitizers.

In the control group, temperature increases were observed at all ultrasound power levels, though these were the lowest among the groups. At 6 W, the control group reached 60.9°C after 5 minutes of ultrasound application.

In the IR780 group, temperature increases exceeded those of the control at every power level. The highest temperature, 70.8°C, was recorded after 5 minutes at 6 W.

The RB group exhibited the greatest temperature elevations, particularly at higher power levels. At 6 W, the temperature peaked at 77.7°C after 5 minutes, the highest across all conditions.

At ultrasound powers of 5 W and 6 W, the control group reached 59.8°C and 60.9°C, respectively. However, both IR780 and RB demonstrated enhanced heat generation, especially at the highest power level (6 W), with the temperature rising significantly beyond that observed in the control group.

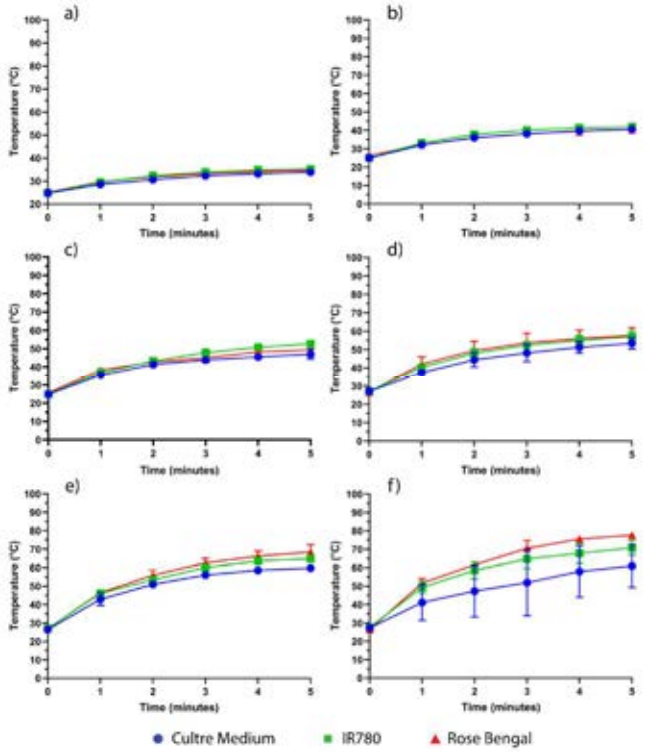


Fig. 3. Temperature increases with the ultrasound exposure of culture medium (blue circle), IR 780 containing culture medium (red triangle), and RB containing culture medium (green square). The power of the ultrasound was set to (a) 1 W, (b) 2 W, (c) 3 W, (d) 4 W, (e) 5 W and (f) 6 W.

IV. Discussion

This study highlights the promising potential of both IR780 and Rose Bengal (RB) as effective sonothermal sensitizers. While ultrasound alone induced a temperature rise in the culture media, the presence of these agents enhanced heat generation, especially at higher ultrasound powers. This ability to amplify thermal effects is critical for sonothermal therapy (STT), which aims to reach temperatures above 50°C in tumors, leading to irreversible cancer cell damage while sparing healthy tissues.

Our results build on existing studies while offering new insights. For instance, while Li et al. demonstrated the sonodynamic effects of IR780, they observed minimal temperature increases during ultrasound treatment in vivo [14]. Similarly, Kim et al. found that the use of a Rose Bengal derivative with pulsed HIFU raised tumor temperatures by only 5°C [15]. As an in-vitro study, our findings demonstrate that both IR780 and RB can increase temperature when exposed to continuous ultrasound, suggesting their potential for STT applications.

In another study, Patel et al. used 45 kHz ultrasound on MDPC-23 cells [16]. Their study observed enhanced cell proliferation and viability with minimal thermal effects, while our findings highlight the effectiveness of megahertz-frequency ultrasound in generating the necessary temperature rise for STT. This difference highlights the critical role that ultrasound frequency and power play in optimizing thermal outcomes for therapeutic applications.

By demonstrating that IR780 and RB can enhance heat generation under ultrasound, this study provides insight into their role in sonothermal therapy. These findings advance our understanding of how these sensitizers can be optimized for cancer treatment, highlighting the effect of ultrasound frequency and power in temperature elevations.

V. Conclusion

The findings of this study highlight that IR780 and Rose Bengal are effective sonothermal sensitizers that are capable of significantly enhancing heat generation under 1 MHz ultrasound. These results not only extend the understanding of the sonothermal potential of these agents but also provide a foundation for future

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