FUNCTIONALIZED NANOPARTICLES MEDIATED HIGH INTENSITY FOCUSED ULTRASOUND (HIFU) ABLATION IN MICE

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INTRODUCTION

High Intensity Focused Ultrasound (HIFU) is a non-invasive procedure that has gathered clinical interest for tumor ablation. Its effectiveness has been demonstrated through *in vitro* and *in vivo* models. The key to treating tumors lies in applying sufficient acoustic power to induce necrosis. However, the challenge arises as high acoustic power may inadvertently cause collateral damage to neighboring tissues, resulting in issues like skin burns, and damage to blood vessels and nerve cells. To address this, external heat absorbers such as nanoparticles have been employed to reduce power requirements and exposure durations.

Numerous researchers have explored the use of nanoparticles to enhance thermal effects and decrease power requirements. For instance, Dibaji et. al. [1] utilized magnetic nanoparticles (mNPs) in tissue-mimicking materials (TMM), while recent studies have shown the efficacy of gold nanoparticles (gNPs) in achieving enhanced thermal effects, both *in vitro* and *in vivo* [2].

In a study by Devarakonda et al. [3], the impact of gNPs during HIFU procedures was investigated for 0% gNPs concentration and 0.125% gNPs concentration. However, there is a possibility of injected gNPs being washed away by the bloodstream, diminishing their presence around the tumor, and hindering the intended temperature enhancement. Considering this, the current study emphasizes the use of gNPs functionalized with antibody fragments (Fab) to ensure they adhere to the tumor after injection.

Ouyang et al. [4] discovered that over 15% of functionalized nanoparticles reach the tumor when administered via tail vein injection, provided the number of injected nanoparticles exceeds 1 trillion, a condition met in the present study. In a previous study from our lab [3], a 0.125% concentration (maximum) of non-functionalized gNPs was used. The current research, however, utilizes 15% of the 0.125% concentration, resulting in a required tumor domain concentration of 0.01875%, considering the use of functionalized nanoparticles.

METHODS

Each NSG mouse in the experiment was injected with 1×10^7 PC3 tumor cells. Mice were then housed and monitored at Cincinnati Children's Hospital and Medical Center (Cincinnati, Ohio). The tumor growth was monitored for $4 - 6$ weeks. The size of the tumor was measured regularly 2-3 days per week. Once the tumor was roughly above 10 cm in both transverse and longitudinal direction, it was deemed suitable for treatment. This was done to ensure that the treatment can be done in at least two zones of a particular tumor.

The functionalized gNPs construct was prepared following several steps reported by Dockery et. al. [5]. A solution of gNP-CO2H/N3 (81 mL, 2.14×10^{13} gNPs/mL) in filtered, pH8 1X PBS was stirred at room temperature in a freshly cleaned 250 mL round bottom flask. 501 mg Fab-DBCO (~10 Fab/gNP from 1 mg/mL stock) was added to this stirring solution. The reactants were stirred for 1 hour at room temperature. After 1 hour the reaction was stopped. The solution was centrifuged (45k x g, 2 hours, 4˚C), and resuspended in 40 mL filtered pH8 1X PBS. This was followed by another centrifugation (45k x g, 2 hours, 4˚C) with resuspension in 6 mL filtered pH8 1X PBS. Subsequently, a final centrifugation step (21 k x g, 1 hour, 4˚C) followed by resuspension in 2 mL (0.62×10^{15} gNP/mL) filtered, pH8 1X PBS was done.

Figure 1: Schematic representation of functionalization of dendronized gNPs with cancer specific targeting antibody fragment, Fab, starting from citrate gNPs.

The resulting concentrated solution was characterized using DLS and UV-Vis spectroscopy. The Z-average of the particle size was 27.49 nm and the maximum absorbance wavelength was 525 nm. The synthesis of intermediate dendrons and materials is described in the literature [6].

Gold nanoparticles (gNPs) were injected 24 h before the treatment. Mice were put in an incubator at 40 ˚C for 1 h before gNPs injection. The solution containing gNPs had a distinct purple color, such that it can be visible around the tumor after the injection.

A clinical MR-HIFU system (Sonalleve V2, Philips Medical Systems, Vantaa, Finland), integrated into 1.5 T (T) whole body scanner (Philips Ingenia, Healthcare, Best the Netherlands) was used for the scanning of the mouse tumors. The MR-HIFU system contains a 256-element phased array HIFU transducer that can be used to focus energy to small volumes within the tumor. The diameter of the array transducer and the operating frequency are 140 mm and 1.2 MHz. The focal spot of the HIFU bean was ~2 mm in radial direction. Further details about the MR-HIFU system can be found in Devarakonda et. al. [2].

On the treatment day, the mice were prepared by shaving around the tumor, applying Nair, and injecting with anesthesia. A set of two mice were placed under the HIFU transducer for the treatment. They were covered with acoustic gel to avoid the formation of bubbles and cavitation. MR images were taken before and after the treatment. The treatment was done first by locating the tumor with test sonication and then applying the assigned power with therapy sonication. The temperature rise using MR Thermometry was observed and recorded for analysis. The mice were kept for 4 h after the treatment under analgesia and then euthanized.

RESULTS

The reference body temperature was taken as 37 °C to calculate the temperature rise in all cases. Figure 2 shows the temperature rise data fitted to a linear profile for two cases: 0 % gNPs and 0.01875 % gNPs concentration cases. It illustrates the temperature rise (°C) using acoustic powers of 30 W and 40 W for gNPs concentration of 0% (hashed blue line) considered as the baseline case. Notably, the temperature rise was approximately 10°C and 17°C for 30 W and 40 W acoustic powers, respectively. When the acoustic power was elevated from 30 W to 40 W with 0% gNPs concentration, the temperature rise increased by 70%. A discernible linear trend is evident, characterized by a slope of 0.41 with R^2 value of 0.99.

Figure 2 also illustrates the temperature rise (°C) at acoustic powers of 40 W and 50 W for gNPs concentration of 0.01875% indicated by solid red line. The temperature rise was around 21°C and 30°C for 40 W and 50 W acoustic powers, respectively. An increase from 40 W to 50 W with 0.01875% gNPs concentration resulted in an increase in temperature rise of 42.8%. A linear trend is observed, featuring a slope of 0.58 with R^2 value of ~ 1. Therefore, the slope of temperature rise is higher in the 0.01875 % gNPs case than in the 0 % gNPs case (Figure 2).

In Figure 3, the temperature rise (°C) is presented for the same acoustic powers of 40 W, but with two different gNPs concentrations: 0% and 0.01875%. Notably, the temperature rose to approximately 18°C with 0% gNPs concentration and 21°C with 0.01875% gNPs concentration at the same 40 W acoustic power. This indicates that the temperature rise at 0.01875% gNPs concentration was 17.3% higher than the 0% gNPs concentration (or baseline case). Figure 3 also shows the temperature map around the focal pixels at acoustic power 40 W for both 0 % and 0.01875 % gNPs concentration.

DISCUSSION

The findings of this study highlight that the temperature rise increases with or without the use of gNPs as acoustic power increases. Also, most importantly, it shows that the temperature rise is higher for the case with gNPs in comparison to the case without gNPs for the same acoustic power. This underscores the significant impact of using functionalized gNPs in enhancing thermal effects at the same acoustic power.

Limitations: The study currently provides results for the 0% gNPs case at acoustic powers of 30 W and 40 W, and for the 0.01875% gNPs case at acoustic powers of 40 W and 50 W. Notably, data for the 0% gNPs with 50 W acoustic power and 0.01875% gNPs with 30 W acoustic power are not presented as these aspects are part of an ongoing study, and their findings will be shared in future presentations. Additionally, the current results are based on two mice in each category, and a more extensive study involving four mice in each category is part of an ongoing study.

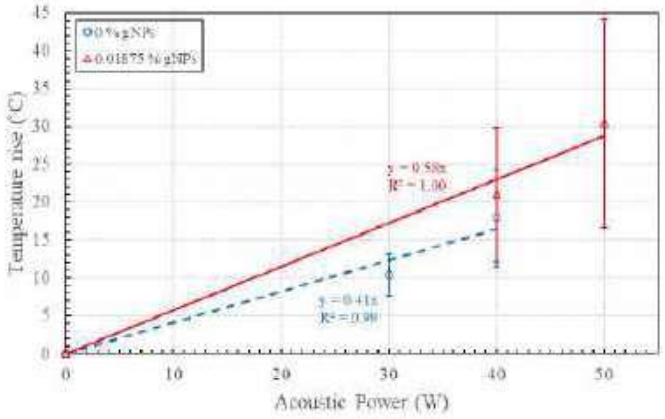

Figure 2: Temperature rise (˚C) with a) 0 % gNPs concentration for acoustic power 30 W and 40 W b) 0.01875 % gNPs concentration for acoustic power 40 W and 50 W

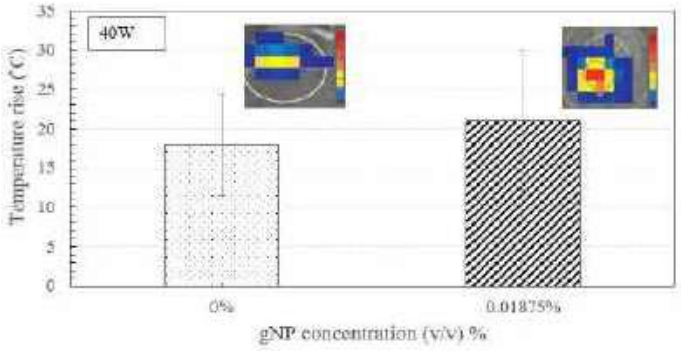

Figure 3: Temperature rise (˚C) for 0 % and 0.01875 % gNPs concentration for acoustic power of 40 W with respective temperature maps

ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation through Grant #NSF CBET 2045234.

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