Progress Report (6 months)

Focused Ultrasound Foundation Research Award for the project titled
"Metastable Perfluorocarbon Nanodroplets for Enhanced HIFU Ablation"

PI: Paul A. Dayton, University of North Carolina at Chapel Hill

Overview:

We are investigating a novel ultrasound contrast agent and its ability to safely enhance high-intensity-focused-ultrasound ablation speed and volume. It has been well established that microbubbles enhance the absorption of acoustic energy thereby enhancing thermal ablation in vivo. Negative effects include skin burns. This metastable nanodroplet has been chemically tuned to convert back into a microbubble at approximately 2 MPa. Most importantly, this new nanodroplet also enhances heating, but does so at the acoustic focus, avoiding surface burns. It may also remain stable in circulation for a longer time period, thereby making it more clinically translatable for hours-long FUS procedures. Combined, these properties should enable focused enhanced ablation. We have been investigating the thermal effects of these agents in pre-clinical studies in vivo.

Summary of Progress to Date:

I. Lower limits of nanodroplet concentration required to enhance ablation.

Our *in vitro* studies have shown that nanodroplets enhance HIFU ablation in tissue-mimicking phantoms. With just 20 seconds of 2, 3, or 4 MPa HIFU no lesions were formed unless nanodroplets were present. The minimum concentration of nanodroplets required to induce lesion was dependent on the intensity of the HIFU. At least 0.005µl of nanodroplets per milliliter were required to induce a lesion with 4MPa of peak negative pressure, whereas 1.0 µl ND/ml were required when only 2 MPa were applied.

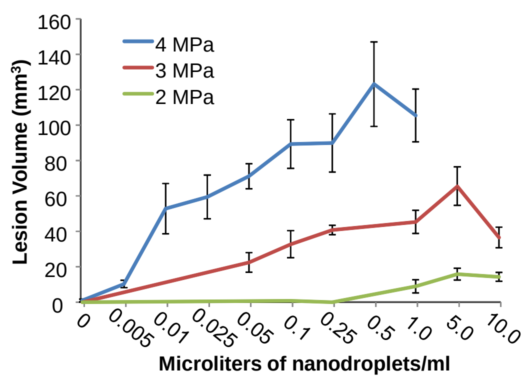

Figure 1: HIFU lesion volume formed in albumin-acrylamide phantoms containing 0-10µl of ND per milliliter. Lesion volume after 20s of 2, 3, or 4 MPa HIFU is plotted as a function of nanodroplet concentration. (mean ±S.D., n≥3)

II. Lowest acoustic threshold required to induce ablation.

Table 1: Lesion formation after HIFU with nanodroplets in rat livers

HIFU Power (W)	HIFU on time (s)	Post ND-injection time (min)	Lesion formed? Yes	No
10	15	-ND		n=1
15	15	-ND		n=1
25	15	-ND		n=2
25	30	-ND	n=2	
5	15	+ND, 2min		n=1
10	15	+ND, 10min		n=1
15	15	+ND, 15min	n=6	
15	15	+ND, 95min	n=4	

In vitro, the lowest acoustic threshold required to induce an ablation lesion in the presence of nanodroplets was 2MPa, however a large, potentially non-clinical, amount was required (see Fig. 1). Using 3 or 4MPa of peak negative pressure resulted in consistent HIFU lesion enhancement with relatively low nanodroplet concentrations (0.05-0.02 µl ND/ml).

In vivo, lesion formation was assessed in rat livers following the application of HIFU between 5-25 Watts using the RKO-100 (FUS Instruments, Toronto). A total volume of 100µl containing a 1:4 dilution of stock nanodroplets (~$1x10^9$ nanodroplets per ml) was injected via the tail vein into each rat. In controls cases, no nanodroplets were injected. HIFU lesions were formed in the livers of animals that had been injected with nanodroplets. HIFU at 15 W (15 s) was applied both shortly after the injection (15 minutes), and 95 minutes after the injection. In order to induce a HIFU ablation lesion in the liver of animals not injected with nandroplets, twice as much time (30 seconds), and significantly more power (25W) was required. In this study where ultrasound was applied for only 15 seconds, at least 15 Watts was required to induce a HIFU lesion when nanodroplets were present.

III. Long term circulation of nanodroplets *in vivo*.

MR-Thermometry was used to assess HIFU induced heating with and without the presence of nanodroplets. Lesions were formed in the livers of animals using only 15W for 15s when nanodroplets had been injected either 15 or 95 minutes earlier. No skin burns were present. The nanodroplets enhanced HIFU heating at all three time points in the livers, resulting in peak temperature rises of 52ºC +/- 14 ºC 95 minutes after their injection. These results indicate that nanodroplets remain active in the liver for at least 95 minutes and could offer ablation enhancement during MRgFUS procedures.

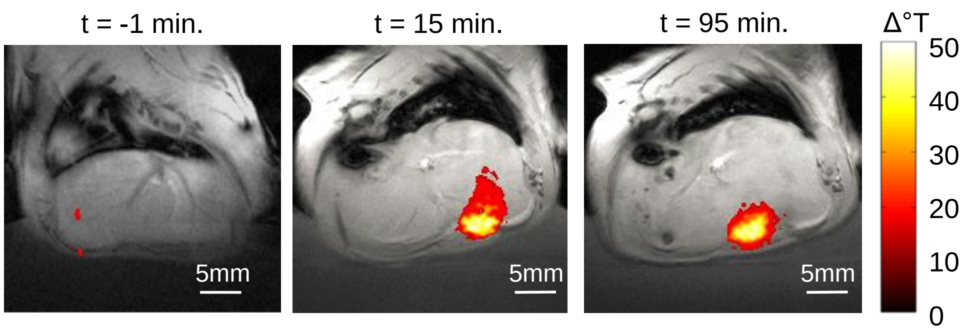

Figure 2: Representative MR cross-sectional images of the rat abdomen comprised primarily of the liver in the bottom half. The corresponding temperature maps have been overlaid onto the greyscale MR images.

IV. Pilot study of nanodroplet-enhanced HIFU ablation in a tumor model.

HIFU enhancement by nanodroplets was assessed in rats bearing flank C6 glimoa tumors. HIFU was applied again by the MR-compatible RKO-100, in the Siemens 3T scanner. Since the tumors were superficial, and the acoustic focus was therefore shallow, an intensity of 12W was applied in order to avoid skin burns at later time points. In separate locations within the tumor, HIFU was applied either before or after the injection of nanodroplets. Without nanodroplets, only minimal heating was observed,

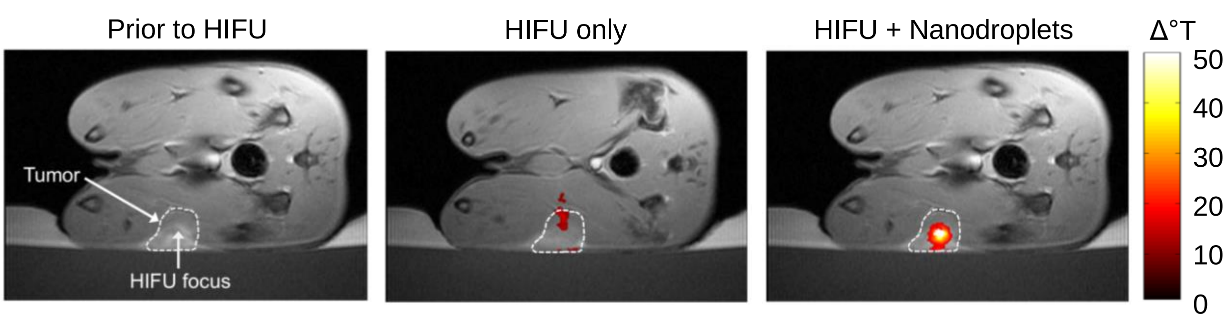

Figure 3: Representative MR cross-sectional images of the rat limbs with C6 glioma flank tumors. The white outline indicates the position of the tumor in each plane. The corresponding temperature maps have been overlaid onto the greyscale MR images. Only minimal heating was observed when 15W HIFU was applied for 15s without a prior injection of nanodroplets (middle panel), but significant enhancement was observed when the same HIFU was applied 15 minutes after nanodroplets had been injected.

and it was located near the acoustic focus (See Fig. 3). The average HIFU and nanodroplet-induced temperature rise observed in the tumors was 34 +/- 5ºC when 15W-HIFU was applied 15 minutes after the injection. Applying HIFU at only 12 W to tumors 95 minutes after injection of the nanodroplets did not result in a significant increase in thermal enhancement compared to agent-free controls. This result may partially be due to the lower acoustic intensity applied and/or the different physiology of the tumors compared to the livers.

Remaining Research:

I. Lower limit of concentration of nanodroplets in vivo. This will be performed in the next 3 months.
II. Acoustic cavitation detection of phase change in phantoms. This study is ongoing and will be performed in the next 4 months.
III. Continued evaluation of nanodroplets in tumors. This study is ongoing and will be performed in the next 4 months.
In addition, studies of HIFU heating in the presence of microbubbles are currently underway.

Summary:

Although enhanced ablation was observed in both the liver and tumors, the effective ablation time was shorter in the tumors. A greater range of timepoints will be evaluated in the future. The liver may preferentially filter the nanodroplets resulting in a higher dose within the liver compared to the tumors. Comparison studies are underway to assess the effective HIFU-ablation lifetime of microbubbles *in vivo*. These mixed-perfluorocarbon nanodroplets may offer a longer-lasting, safe method of ablation enhancement by HIFU, as demonstrated by the increased thermal deposition combined with a reduced pressure requirement to induce ablation.

Publications from the project include:

- Puett, C, Phillips, LC, Sheeran, PS, Dayton, PA, “In vitro parameter optimization for spatial control of focused ultrasound ablation when using low boiling point phase-change nanoemulsions.” Journal of Therapeutic Ultrasound, vol. 1-16, pp.1-13, 2013.

Oral presentations related to the funded project include:

- Phillips, LC, Puett, C, Sheeran, PS, Timbie, KF, Price, RJ, Miller, GW, Dayton, PA, “Dual perfluorocarbon nanodroplets enhance high intensity focused ultrasound heating and extend therapeutic window *in vivo*.” Acoustical Society of America Meeting (Dec. 2013).

- Phase-shift Perfluorocarbon Nanodroplets for Enhanced High Intensity Focused Ultrasound –UVA Focused Ultrasound Foundation Center Evening Symposium, Charlottesville, VA (Sept. 2013).

- Phillips, LC, Puett, C, Sheeran PS, Timbie, KF, Price, RJ, PS, Miller, GW, Dayton, PA, “Enhanced In Vivo High Intensity Focused Ultrasound Alabation via Phase-shift Nanodroplets Compared to Microbubbles” IEEE-International Ultrasonics Symposium (July 2013).