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Image-guided focused ultrasound therapy system and method for improved anticancer drug delivery

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***Abstract*—Vascular abnormalities in a complex tumor microenvironment is one of the major challenges for effective drug delivery and cancer treatment. It has been well established that noninvasive focused ultrasound (FUS) combined with a microbubble (MB) contrast agent can safely and reversibly increase the permeability of blood vessel walls, thereby temporarily allowing anticancer agents to pass through them and into the tumor tissue. Termed FUS-mediated drug delivery, most previous studies have used a single focused US beam for cancer treatment in small animal models, which limited the ability to treat the entire tumor burden. To that end, the goal of this research was to reveal the effectiveness of tumor treatment when using multi-FUS system and method. An infrared dye (IR-780) functioned as a surrogate chemotherapeutic drug and allowed detection in live animals. Athymic nude female mice implanted with MDA-MB-231 breast cancer cells were used to evaluate both single FUS and multi-FUS therapeutic strategies. Each animal was injected in the tail vein with a bolus mixture of MB (2.3×10^7 Definity, Lantheus Medical Imaging) and IR-780 dye (50 µg). During US therapy, a custom pulsed sequence was applied using an image-guided FUS system (HIFUPlex-06, Verasonics Inc) for a duration of 10 min in the selected zone of the tumor. Animals were imaged using a whole-body optical imaging system (Pearl Trilogy, LI-COR Biosciences) and accumulated IR-780 dye was quantified up to 48 h after application of FUS-mediated drug delivery. After euthanasia, IR-780 dye was also quantified from homogenized tumor tissue samples. Overall, preliminary results showed that the multi-FUS therapy approach significantly increased drug uptake (increased by about 71 % at 48 h) in the targeted tumor tissue compared to the single FUS method.**

Keywords—cancer; drug delivery systems; image-guided therapy; microbubbles; ultrasound

I. INTRODUCTION

Abnormal tumor vasculature and the resulting microenvironment are major barriers for optimal chemotherapeutic drug delivery to the tumor space and cancer treatment [1] [2]. Increasing the overall drug dose is generally not an option to overcome these physical barriers due to concerns with systemic toxicity and patient tolerance. It has been well established that noninvasive focused ultrasound (FUS) can safely and reversibly increase the vascular permeability and deliver an intravenously injected drug to a target site [3] [4]. Exposure of microbubble (MB) contrast agents with low-intensity FUS can be used to better control this process and reduce the risk of tissue damage [5]. Ultrasound (US) at lower acoustic pressures (order of a few hundred kilopascals, kPa) can cause circulating MBs to volumetrically expand and contract in response to the compression and rarefaction phases of the pulsed US waves, respectively. This process of stable cavitation results in enhanced microvascular permeabilization and improved localized drug deposition in tumor [6].

Several recent papers by our group and others have reported promising results from both low-intensity unfocused and FUS-mediated drug delivery in small animal imaging as a method to enhance cancer treatment [3] [4] [7] [8]. Recently, Kotopoulis et al. [9] showed the ability and efficacy of FUS-mediated gemcitabine drug delivery in a clinical setting and using commercially available MBs. This study proved that US therapy can positively impact patient survival by reducing tumor size and growth. The importance of this pioneering study is that it represents the first time that US-mediated drug delivery has been used in human patients to improve cancer treatment.

In addition to improved cancer-based treatment, FUS-mediated drug delivery also helps to temporarily enhance gene therapy vectors [6] [10] and to locally induce a transient disruption of the blood-brain barrier (BBB) to facilitate targeted drug delivery [11] [12]. These studies predominately used a single element US transducer and/or single focal zone to help improve microvascular permeabilization. In this study, we introduce a multi-FUS system and method to further improve the therapeutic process and compare to tumor treatment using a single FUS neighborhood. The overarching goal was to investigate any improved tumor treatment in space using proposed multi-FUS method and target tissue uptake of an infrared dye in live animals.

II. MATERIALS AND METHODS

A. Animal preparation

Animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas Dallas. Six-week-old athymic female nude mice (Charles River Laboratories) were implanted subcutaneously in the mammary fat pad with one million MDA-MB-231 breast cancer cells. The implanted tumors were allowed to grow for approximately four weeks. When tumor size reached a target volume (approximately 450 mm^3 as measured using digital calipers), animals were randomly sorted into two treatment groups, namely, single or multi-FUS (N = 3 per group). Mice were anesthetized using isoflurane and kept on a heating pad to maintain core temperature. A catheter was

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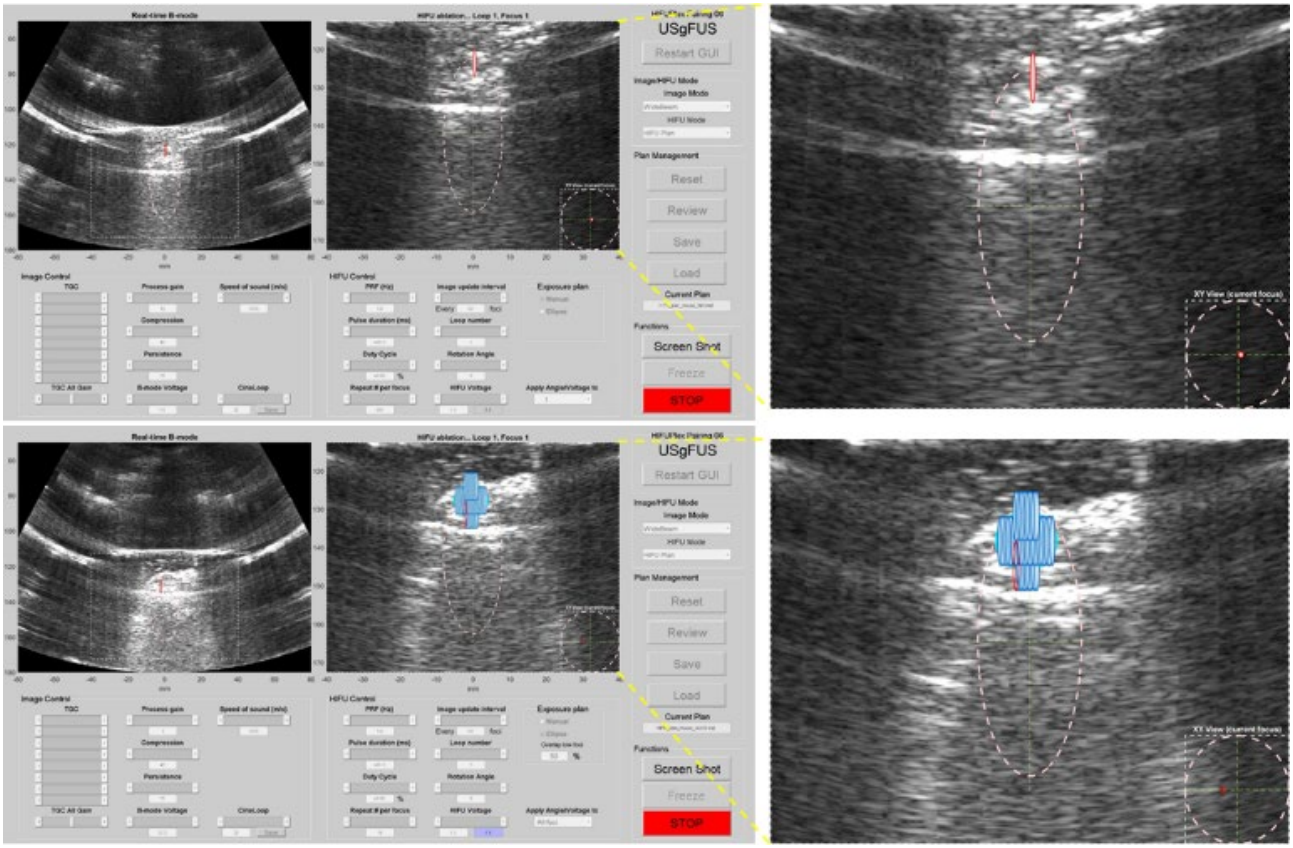

Fig. 1. Graphical user interface (GUI) screenshots used for ultrasound (US) imaging guidance and application of single focused US (FUS) (top) and multi–FUS (bottom) treatment.

inserted into the tail vein to prepare them for exposure to image-guided FUS therapy.

B. Ultrasound therapy

A 3-dimensional (3D) US image-guided FUS therapy system (HIFUPlex-06) was used for this study. This system consisted of a programmable US research scanner (Vantage 256, Verasonics Inc) equipped with a dual transducer configuration (Sonic Concepts). These co-registered transducers included a focused transducer for application of US therapy and an US transducer for interleaved imaging and guidance. Both the imaging and therapeutic transducers were 128-element arrays with center frequencies of 3.5 and 2.0 MHz, respectively. The latter is a concentric array and allows for beam steering in 3D space. A graphical user interface (GUI) on the Vantage system allows visualization of the target tissue and control of therapy with protocol design and preview. The therapeutic US transducer exposure involved a 400 ms pulse duration, 10 min duration of exposure, 40% duty cycle, and a mechanical index (MI) of 0.5. The single FUS therapy repeats at the specified focal spot location, whereas multi-FUS therapy uses multiple transmissions at a particular focal spot and then is moved to the next planned focal spot. The multiple transmissions (i.e., repetition per focus) were calculated based on the number of focal spots used in the therapy. The acoustic output from the therapeutic transducer was calibrated using a scanning tank for hydrophone-based measurements (AIMS III, Onda Corp). Samples of MB contrast agent (2.3 x 10^7 MBs, Lantheus Medical Imaging) and IR-780 dye (50 µg) were mixed and diluted with saline before being injected slowly through the placed catheter. Immediately thereafter, image-guided FUS therapy was applied to the tumor tissue after coupling the water-backed polystyrene-coated acoustic aperture with US transmission gel (Aquasonic 100, Parker Laboratories).

C. Optical imaging

Optical images of IR-780 dye accumulation in the targeted cancer tissue were acquired in live animals (Pearl Trilogy, LI-COR Biosciences) at baseline and then again at 0.1, 24, 48 h after FUS-mediated drug delivery [13]. The mice were captured by both white light and fluorescence imaging. The fluorescence imaging was operated using the 800-nm channel with 785 nm excitation and 820 nm emission filters. Fluorescent signal intensity was measured within a user-defined region-of-interest (ROI) using the vendor software (Image Studio Software, LI-COR Biosciences). ROIs were manually drawn around each tumor using the guidance of the white light image. All measurements were first normalized by background signal and then by ROI pixel count to quantify mean intratumoral fluorescence activity, which was a surrogate measure of drug delivery and accumulation.

D. Tumor tissue dye extraction

Tumors were excised after optical imaging at 48 h following animal euthanization. Tumors were weighted, cut into smaller pieces, and rinsed with saline. These pieces were mixed with 1

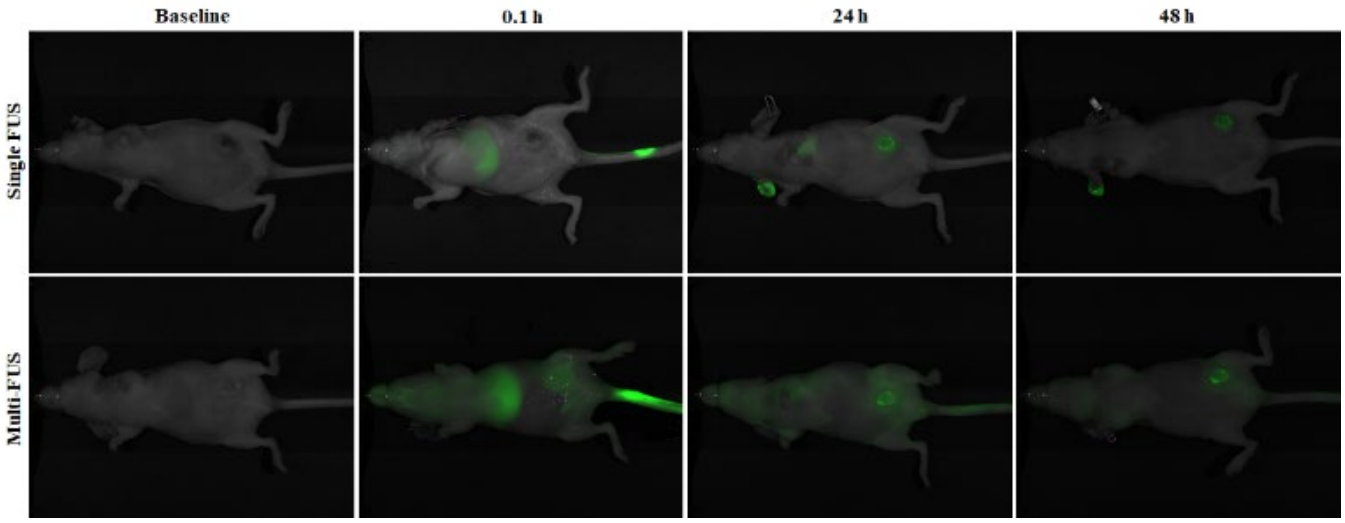

Fig. 2. Representative *in vivo* optical images of cancer-bearing animals following injection of a surrogate drug (IR 780 dye) and application of single (top) or multi-FUS (bottom) therapy. Fluorescence images were acquired at baseline before application of US treatment and again at 0.1, 24, and 48 h.

mL of radioimmunoprecipitation assay buffer and transferred to 2 mL tubes containing ceramic beads. The buffer had 50 mM The buffer had 50 mM Tris-base (pH 7.4), 150 mM sodium chloride (NaCl), 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (SDS) as we described previously [14]. Tubes then underwent high force homogenization (Bead Mill 4 Homogenizer, Fisher Scientific) for IR-780 dye extraction. After centrifugation at 2000 g for 10 min (repeated twice), the supernatants were transferred to a 96-well black plate (200 µL per well). Controls of known IR-780 concentration were run along with the supernatants of the tissue samples. Each tissue sample was measured in triplicate. The fluorescence signal from each well was quantified by a microplate reader (Synergy H4, BioTek) with optical excitation and emission set at 780 nm and 820 nm, respectively. IR-780 dye uptake of the tumor was calculated and represented as the percentage of dye retained in the tumor out of total dye injected into the animal.

III. RESULTS

Fig. 1 shows the FUS therapy software and control interface used in single and multi-FUS therapy. The screenshots were saved while performing the therapies from the HIFUPlex GUI. The GUI has two main windows, namely, US imaging and treatment plan visualization. The US imaging window displays the B-mode image and the parameters that control this image are active in the lower left quadrant of the screen. The therapy screen is on the right side of GUI and it allows for planning using a user and graphical-based method. The therapy can be planned and saved before the treatment, which consists of a sequence of focused and spatially distributed US exposures. The focal spots are ellipsoidal in shape and many can be placed within a dotted larger ellipsoid either manually or automatically (by drawing the region around a tumor). The focal spots automatically appear over the tumor region based on the overlap specified; the overlap was set to 50% in this study. The focal spots are color-coded such that the planned focal spot with cyan, ongoing therapy focal spot with red, and already exposed therapy focal spot with yellow color. One can visualize the progression of FUS therapy and the movement of the different focal spots in the real-time US images.

Optical images of intratumoral fluorescence tracer uptake were acquired at 0.1, 24, and 48 h after exposure to single or multi-FUS-mediated drug delivery. Presented in Fig. 2, review of these images clearly reveals improvement in IR-780 dye accumulation in tumor tissue for the multi-FUS therapy case compared to the single FUS strategy. Quantification of the fluorescence signal from these images is summarized in Fig. 3. Compared to data from tumors exposed to single FUS therapy, temporal measurements demonstrate a nearly 108% increase in fluorescence tracer accumulation at 0.1 h after tumor treatment using multi-FUS followed by increases of 92.5% and 71.1% at 24 and 48 h, respectively.

Tumor samples were processed to extract the IR-780 dye, which provides an additional measure of drug delivery and accumulation. The fluorescence signal from the extracted dye reveals that there is a significant improvement in intratumoral

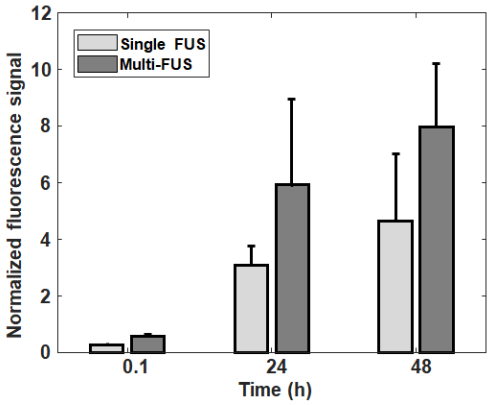

Fig. 3. Summary of *in vivo* optical imaging results from cancer-bearing animals at baseline and at 0.1, 24, and 48 h after exposure to single or multi-FUS-mediated drug delivery. Note the improved IR-780 dye uptake for the multi-FUS case.

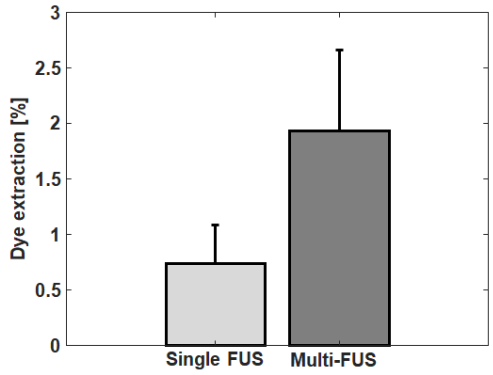

Fig. 4. Comparison of IR-780 dye extraction data obtained from excised tumor samples at 48 h after exposure to single and multi-FUS therapy.

accumulation after exposure to multi-FUS therapy. Specifically, multi-FUS therapy exhibited a 169.8% increase in fluorescence signal intensity. The results indicate that the dye extracted from excised tumor samples is highly correlated with the results from the *in vivo* optical measurements obtained immediately prior to euthanasia.

IV. DISCUSSIONS AND CONCLUSIONS

Previous studies have shown that MBs exposed to single FUS can considerably improve drug or gene delivery to the targeted tissue space. However, use of a single US focus only allows MB interaction and US therapy delivery within the beamwidth of the focal spot. Consequently, this strategy fails to treat any tissue outside the US focus. One may manually move the transducer to cover the entire targeted tissue space, but this approach has additional challenges like uniform treatment coverage. Alternatively, we introduced the multi-FUS concept for the US treatment of cancer. This new FUS-mediated drug delivery system and method helps to control and distribute US energy over an entire tumor space. Our preliminary results clearly demonstrate the advantage of using a multi-FUS approach over single FUS. Improved IR-780 dye accumulation in tumor tissue was confirmed using both optical imaging of live animals and from dye extraction of excised tissue samples.

Overall, these preliminary results are encouraging, and additional studies are required to understand and further optimize the FUS therapy settings. The therapy settings include overlap between the focuses and repetition per focus along with the other settings like MI, pulse repetition frequency (PRF), pulse duration. While this study only evaluated the multi-FUS strategy in a single plane of a tumor region, the treatment protocol can be extended to 3D space for application of FUS-mediated drug delivery to the entire tumor burden.

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