FUSF Final report

Doxorubicin is a potent anticancer drug used extensively in the treatment of a variety of cancers, including breast and ovarian. Due to adverse side effects, most notably toxicity to the heart, the dose of doxorubicin administered to patients is severely limited. Modifying the pharmacological profile of doxorubicin can reduce these side effects while improving its effectiveness against tumors. To accomplish this goal, we have developed a novel polymer-modified temperature-sensitive liposome (**pTSL**) for localized drug delivery . Systemically administered pTSL accumulates preferentially within solid tumors, and encapsulated doxorubicin can be released locally by heating the tumor specifically with magnetic resonance-guided focused ultrasound (MRgFUS). To further develop the novel delivery approach, we proposed a method to monitor release of doxorubicin and assess the efficacy of doxorubicin delivered via pTSL against breast cancer.

Specific Aim 1: Test the correlation between HIFU-triggered release of manganese and DOX from TSL *in vivo*
Hypothesis: HIFU-triggered release of manganese and DOX from TSL correlates strongly

When heated beyond a threshold temperature, the pTSL become leaky and can release encapsulated biologics within minutes. We proposed to monitor the increase in pTSL leakiness by coencapsulating manganese with doxorubicin. Manganese has a very weak MR signal when packaged within liposomes, which is dramatically increased upon triggered release. In preliminary tests, we successfully loaded manganese into pTSL and detected a quantifiable increase in MR intensity upon triggered release in heated water baths. However, we were not able to detect a quantifiable change in MR signal intensity in solid tumors with our MR scanner. Because the intensity of the MR signal from manganese is a function of concentration, we speculate that the manganese concentration released from the pTSL was too low to produce a detectable signal within our 4.7 Tesla MR scanner. This limitation can be addressed by using a more powerful MR scanner or increasing the concentration of manganese loaded into the pTSL. Unfortunatley, coencapsulating doxorubicin limits the loading capacity for manganese within pTSL, and a more powerful MR scanner was not available for animal experiments. Consequently, we were not able to move forward on this aim. However, other groups have demonstrated the feasibility of this approach for detecting ultrasound-triggered release of liposomal contents with MRI .

Specific Aim 2: Evaluate the efficacy of MRI-guided HIFU-triggered DOX release against tumor growth.
Hypothesis 2: MRI-guided HIFU-triggered DOX release from TSL is more efficacious than systemic injections of free DOX.

The pTSL that has been developed by the lab is very unique in that rapid drug release is possible, particularly when the liposome is heated quickly (< 10 min). As a result, the thermal dose required for drug release is dramatically reduced compared to traditional thermosensitive liposome (TTSL) formulation. As shown in Figure 1, the thermal dose required for 50% DOX release from pTSL was 100-fold less that what was required for DOX release from traditional thermal liposome formulations. Based upon this result, we speculated that DOX delivered to implanted tumors would be more efficacious than free

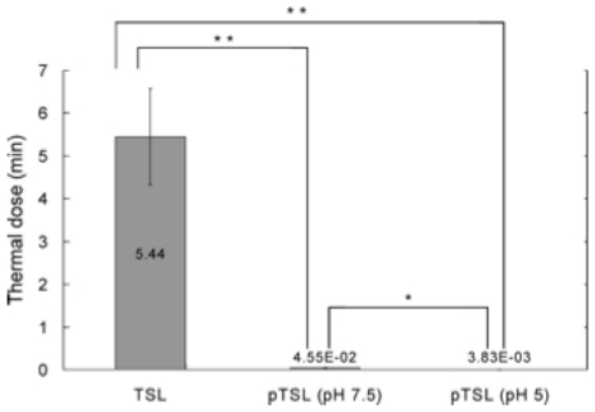

Figure 1. Thermal doses (equivalent minutes at 43°C) required for 50% drug release for TSL (n=4), pTSL at pH 7.5 (n=4), and pTSL at pH 5 (n=4) in 20 mM HEPES. Differences across all groups were statistically significant (* $p<0.001$, ** $p<0.0001$, Student's T-test).

DOX or DOX packaged in non-thermosensitive liposomes (NTSL) administered systemically. Furthermore, we noted a pH-dependence of DOX release from pTSL. This is due to the incorporation of propyl acrylic acid in the copolymer, which is known to have membrane-disruptive capabilities in mildly acidic conditions (pH < 6.5).

The vast majority of *in vivo* studies conducted previously on heat-triggered drug release from thermosensitive carriers used heated water baths or interstitial energy sources. However, this heating approach limits the location and type of tumor that can be heated. To address these limitations, we proposed the use of MR-guided high-intensity focused ultrasound for monitored and controlled heating of solid tumors. First and foremost, we identified the acoustic parameters (acoustic power, pulse length, duty cycle) required to heat solid perfused tumors and sustain the elevated temperature. As shown in Figure 2, we were able to heat implanted tumors and, more importantly, hold the temperature between 42-44^{o}C for five minutes. This provides a thermal dose that was shown in our *in vitro* studies to be effective for triggering release of at least 50% of DOX encapsulated within pTSLs (Figure 1).

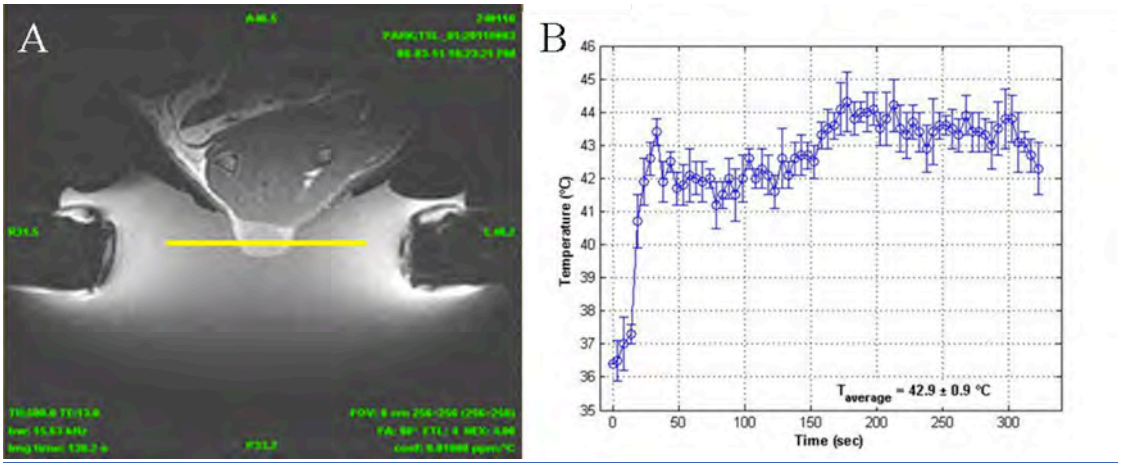

Figure 2. MR-guided FUS (8 elements sector vortex, 1.15 MHz) was used to heat implanted tumors. (A) MR-image of solid tumor during FUS exposure. Yellow line represents plane at which MR thermometry quantifies temperature elevations as a function of time, with trace shown in (B).

To assess the level of DOX release *in vivo*, tumor-burdened rats were injected with TSL or pTSL loaded with DOX ([DOX] = 5 mg/kg). After waiting six hours for particle accumulation, MRgHIFU was used to heat tumors in rats injected with TSL or pTSLto 40^{o}C or 43^{o}C for five minutes. Rats from each treatment group were sacrificed following insonation, and tumors were harvested and sliced. Fluorescent images of tumor sections were acquired to examine the spatial distribution and amount of DOX released by MRgHIFU-mediated heating. As shown in Figure 3, DOX was released from pTSL to a greater extent than from TSL during MRgHIFU-mediated heating. We detected more unencapsulated DOX in tumors that were harvested from rats injected before MRgHIFU with pTSL than TSL, which suggests that DOX was released more efficiently from pTSL than TSL. To examine DOX release more quantitatively, tumors were harvested from sacrificed rats after treatment. Homogenized tumor tissue samples in all treatment groups demonstrated measurable levels of DOX, as determined by fluorescence. Animals given PTSL (5 mg/kg) and FUS (43°C) demonstrated higher levels of drug than animals given TSL (5 mg/kg) and FUS (43°C) (7.9 ug DOX/g tissue to 5.8 ug/g, respectively). This difference was not statistically significant, likely due to extraction of both released and unreleased DOX from the homogenates.

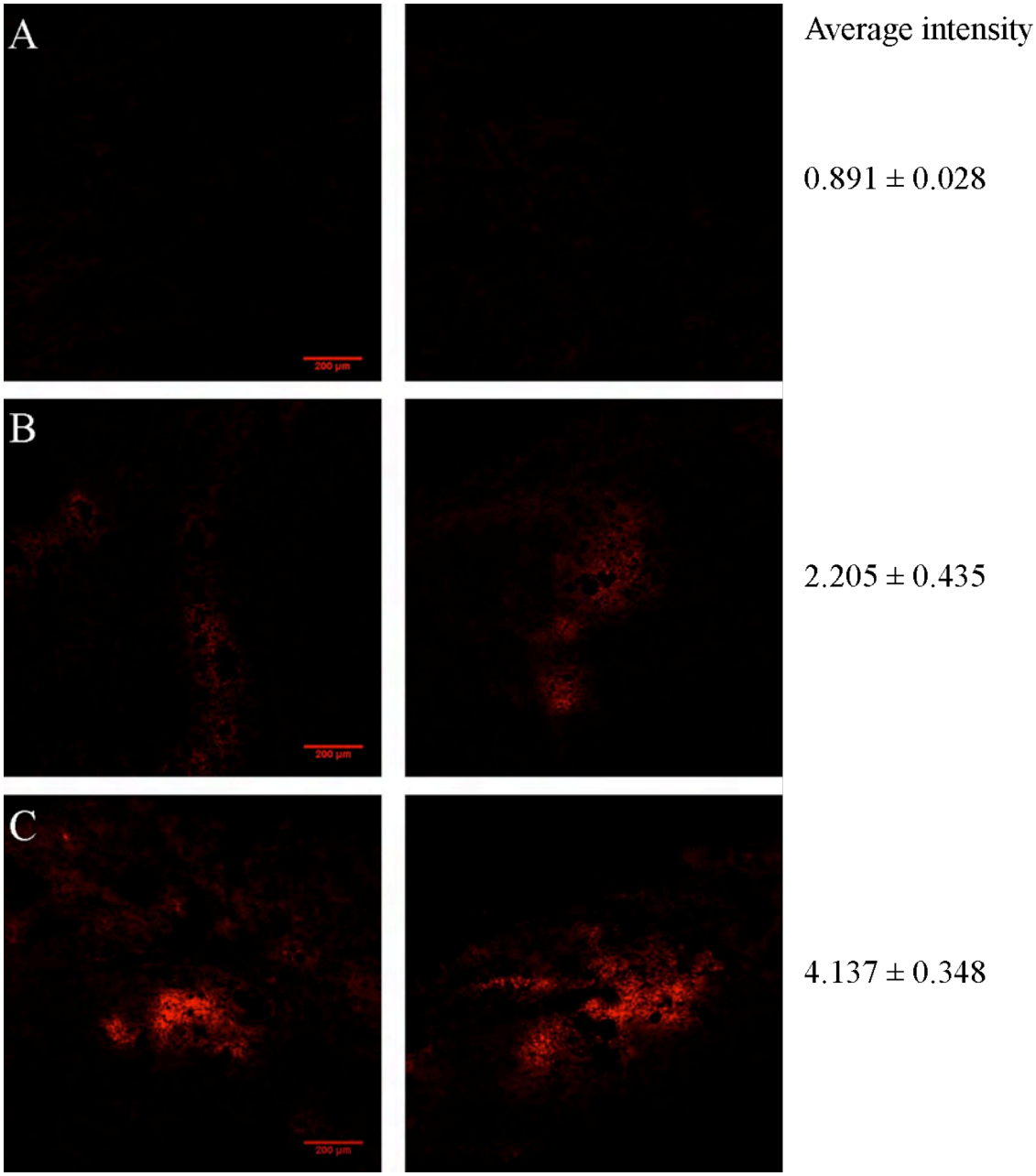

Figure 3. Fluorescent micrographs of tumor sections administered (A) no treatment; (B) TSL-DOX (5 mg/kg) with FUS exposure (43°C, 5 min); and (C) PTSL-DOX (5 mg/kg) with FUS exposure (43°C, 5 min). Average intensities in the images in ImageJ were calculated and presented.

Finally, the response of tumors to triggered release of DOX from liposomes was investigated. Tumor-burdened rats were injected with TSL (5mg/kg) or pTSL (3 mg/kg or 5 mg/kg), and MRgHIFU was used to heat tumors to 40°C or 43°C for 5 minutes to trigger DOX release. As a control, tumor-burdened rats were treated with free DOX (5 mg/kg) administered systemically. The administration of DOX-loaded TSL in combination with FUS exposure (43°C) resulted in significantly smaller tumor volumes when compared to untreated control, with tumors reaching ~3300 mm^3 at day 8 ($p < 0.0005$). However, tumors in the TSL (5 mg/kg) + FUS (43°C) group were significantly larger than those of the free DOX group ($p = 0.014$). This suggests that the increased accumulation of DOX at the tumor – which presumably occurs via encapsulation of the drug into TSL and exploitation of the EPR effect – is negated by the fairly slow

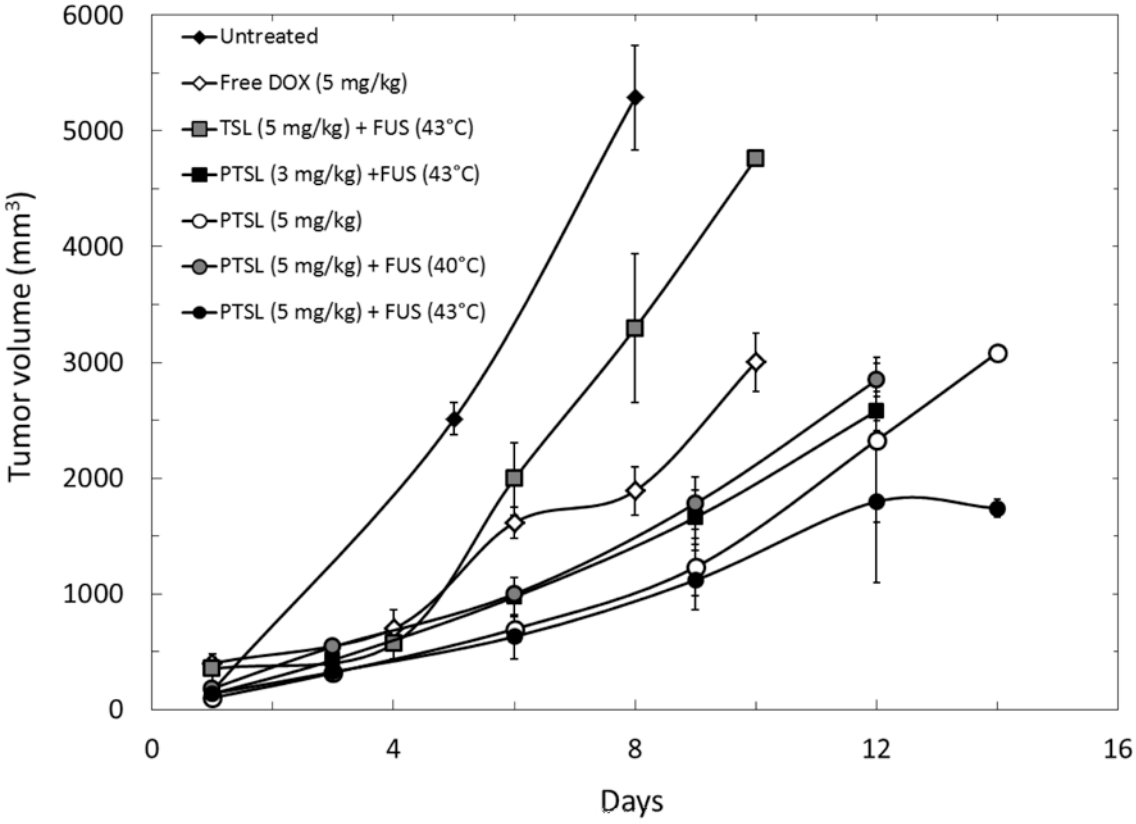

Figure 4. Tumor volume growth curves. Treatment groups were as follows: untreated, free DOX (5 mg/kg), TSL-DOX (5 mg/kg) + FUS (43°C), pTSL-DOX (3 mg/kg) + FUS (43°C), pTSL-DOX (5 mg/kg) without FUS, pTSL-DOX (5 mg/kg) + FUS (40°C), and pTSL-DOX (5 mg/kg) + FUS (43°C). Data shown are averages ± SD for a minimum of n=3 replicates (except for the untreated group, for which n=2). All curves that do not have data out to 14 days indicate animals were sacrificed at day of last timepoint due to excessive tumor volume and necrosis.

drug release kinetics of the formulation at 43°C. As shown in Figure 3, significantly less DOX was released from TSL when tumors were heated for 5 minutes. Published studies suggest that TSL need to be heated for at least 30 minutes in order to release more than 40% of encapsulated DOX [5]. Thus, in this study, the amount of DOX released during MRgHIFU was sublethal and subsequent DOX release over several days was too slow to prove effective.

When compared with the combined TSL-DOX (5 mg/kg) and FUS (43°C, 5 min) treatment, pTSL-DOX treatments with: (a) identical FUS exposure but reduced dose (3 mg/kg); (b) identical dose (5 mg/kg) but lower FUS exposure (40°C, 5 min); and (c) identical dose (5 mg/kg) but no applied FUS all demonstrated

significant reductions in tumor volume. While tumors treated with pTSL-DOX (5 mg/kg) in the absence of FUS grew more slowly than untreated tumors, it was the administration of pTSL in combination with FUS (43°C, 5 min) where the greatest reduction in tumor growth was observed. At day 14, tumors were ~1700 mm^3 in volume, compared to tumors in the untreated control group (~5000 mm^3 at day 8), TSL (~4500 mm^3 at day 10), free DOX (~3000 mm^3 at day 10), and pTSL without FUS (~3000 mm^3 at day 14). At day 14, the difference between the pTSL with and without FUS was statistically significant with $p = 0.002$ (Student's t-test). This demonstrates that the combination of both the acidic tumor microenvironment with heating from externally applied FUS is required to provide the highest concentration of drug at the tumor and thus the most effective clinical response. Furthermore these results indicate that the application of a single, short (5 min) FUS exposure is able to significantly slow tumor growth, and that tumor growth may be further inhibited with multiple administrations of pTSL and/or FUS.

In summary, funding from FUSF has supported the development of a polymer-modified liposome that is srensitive to both heat and acidity. This is the first lipid-based nanoparticle with this dual sensitivity that can be loaded efficiently and stably entrap doxorubicin in serum-containing media. Furthermore, our labs successfully identified acoustic parameters necessary for maintaining solid perfused tumors at an elevated temperature and sufficient for triggered drug release from thermosensitive liposomes. The results of our studies suggest that our polymer-modified thermosenstivie liposome (pTSL) compared to traditional thermosensitive liposomes: (a) releases a greater amount of drug at the tumor in response to heating, thereby reducing the required drug dose; (b) has a lower onset temperature for drug release (T_m), thereby reducing the required thermal dose and improving the safety profile of these formulations; and (c) possesses a pH-sensitivity that responds to the slightly acidic tumor microenvironment, promoting drug release in the absence of externally applied heating.

Products

The results of this FUSF-funded project were shared with the scientific community via a combination of publications, abstracts, and presentations.

Publications

Ta T, Convertine A, Reyes C, Stayton P, Porter T. Thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers for triggered release of Doxorubicin, *Biomacromolecules* (*2010*), *Vol. 11, 1915-1920.* (listed as one of the most accessed articles for the journal in 2010)

Ta T, Park EJ, Zhang, YZ, Bartolak-Suki E, McDannold NJ, Porter TM. Localized delivery of doxorubicin by polymer-modified thermosensitive liposomes: an *in vivo* study for cancer treatment, *in preparation*

Abstracts

Ta T, Park EJ, MacDannold N, Porter TM. Ultrasound-triggered release of doxorubicin from thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers for cancer therapy. *Proceedings of the 3rd International Symposium on Focused Ultrasound (2012).*

Ta T, Park EJ, MacDannold N, Porter TM. Combination of magnetic resonance-guided focused ultrasound and polymer-modified thermosenstivie liposomes for cancer therapy, *J Acoust Soc Am (2012), Vol. 131, No. 4, 3248.*

Presentations

Ta T, Park EJ, MacDannold N, Porter TM. Ultrasound-triggered release of doxorubicin from thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers for cancer therapy. 3rd International Symposium on Focused Ultrasound, Bethesda, MD, October 14-17, 2012.

Ta T, Park EJ, MacDannold N, Porter TM. Thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers for focused ultrasound-triggered release of doxorubicin. 9th Interventional MRI Symposium, Boston, MA September 22-23, 2012.

Ta T, Park EJ, MacDannold N, Porter TM. Combination of magnetic resonance-guided focused ultrasound and polymer-modified thermosenstivie liposomes for cancer therapy, Acoustics 2012 Hong Kong, The People's Republic of China, May 13-18, 2012.

Stimuli-responsive colloidal particles for diagnostic and therapeutic applications. Department of Biomedical Engineering, Worcester Polytechnic Institute, December 14, 2011.