Project Title: MRI-guided localized delivery of chemotherapy using temperature-sensitive liposomes and high intensity focused ultrasound
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Progress Report
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The overall goal of the project is investigate the efficacy of MRI-guided high intensity focused ultrasound for triggering release of doxorubicin (DOX) from temperature-sensitive liposomes. Specifically, we aim to investigate the correlation between release of DOX and the MRI contrast agent manganese and thermal dose applied via ultrasound-mediated heating. While we have made substantial progress on a new formulation of the temperature-sensitive liposome (TSL), we suffered a major setback to the *in vivo* studies. More details are provided below.

Progress on temperature-sensitive liposomes

TSL can require heating to at least 43°C for 30 minutes in order to release more than 50% of encapsulated DOX. Heating tissue for this length of time will result in a thermal dose that can lead to the onset of necrosis. To address this issue, we have modified TSL with a copolymer consisting of the temperature-sensitive monomer *N*-isopropylacrylamide (NIPAAm) and the pH-sensitive monomer propylacrylic acid (PAA). The copolymer – p(NIPAAm-co-PAA) becomes hydrophobic in mildly hyperthermic and/or acidic conditions. The copolymer is end-terminated with a hydrophobic moiety, which facilitates the absorption of the copolymer to liposomal shells. In preliminary studies of the effect of NIPAAm-co-PAA on the temperature-sensitivity of the liposomes, we measured a reduction in the threshold temperature for triggered release of DOX (Figure 1). This effect was enhanced at pH 5, demonstrating the pH-sensitivity of the copolymer. More importantly, polymer-modified

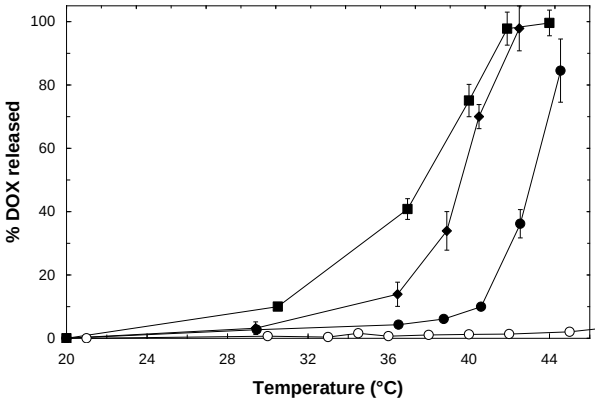

Figure 1. DOX release **in 20 mM HEPES** as a function of temperature (**5 min incubations**), for NTSL (○, n=4), traditional TSL (●, n=4), pTSL at pH 7.5 (◆, n=4), and pTSL at pH 5 (■, n=4).

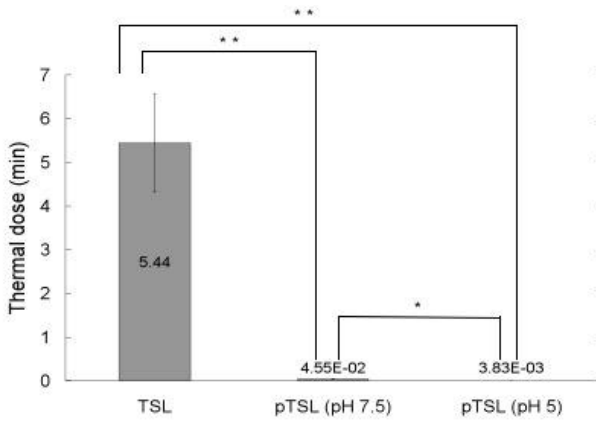

Figure 2. Thermal doses (equivalent minutes at 43°C) required for 50% drug release for TSL (n=4), pTSL at pH 7.5 (n=4), and pTSL at pH 5 (n=4) in 20 mM HEPES. Differences across all groups were statistically significant (* p<0.001, ** p<0.0001, Student's T-test).

liposomes (pTSL) required a significantly lower thermal dose than conventional for triggered release of 50% of encapsulated DOX at pH 7.5 (Figure 2). Currently, we are conducting studies to test the stability and release profile of pTSL in serum.

Setbacks to start of *in vivo* studies

The MRI animal scanner we planned to use for this study was discarded by Harvard Medical School weeks after funding was awarded by FUSF. We have identified an MRI animal scanner at Beth Israel Deaconess Medical Center (BIDMC) to conduct our experiments. However, BIDMC required our personnel to undergo extensive safety and animal handling training for their facilities, which was completed at the end of May. Because MRI is a critical component for our project, we were able to start ordering animals until June. We have begun tumor implantation in nude rats, and expect to begin drug delivery experiments in July. Due to these setbacks, we request a no-cost extension for this project to April 30, 2011.