Optimization of *In vivo* Ultrasound Parameters for Efficient Microbubble Mediated Drug Delivery

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Background, Motivation and Objective

We have previously shown that microbubble-mediated delivery of miRNA-loaded nanoparticles to cancer is a promising treatment approach to sensitize hepatocellular carcinoma (HCC) to chemotherapy drugs. Selection of ultrasound (US) parameters used for such drug delivery, including pressure, pulse repetition frequency (PRF), and exposure time, is often based on phantom studies or previously reported in the literature, and sometimes forgoes rationale for crucial US parameters. Therefore, we performed an *in vivo* study to directly optimize US parameters for miRNA delivery based on the physiological conditions of our animal model.

Statement of Contribution/Methods

A Verasonics Vantage 256 scanner and L11-5 probe capable of US-guided drug delivery were used in an orthotopic mouse model of HCC. Semiconducting polymer nanoparticles (SPNs) were used as a model nanoparticle and co-injected with microbubbles (MB). Perfusion curves were obtained after applying a single therapeutic pulse over a range of voltages to destroy the MBs. The MB re-perfusion time was measured and used to estimate the optimal PRF and voltage for the model. To optimize the drug delivery time, US therapy with the newly optimized PRF and voltage was applied for 10, 20, 30, 50, 75 and 100 sec, and mice were euthanized after 24h. Fluorescent microscopy was used to obtain the distribution of SPNs in the tumor and to select the optimal drug delivery time. Efficiency of US-guided drug delivery with optimized and non-optimized parameters was compared.

Results/Discussion

Based on the perfusion curves (Fig. 1, a), perfusion time was 1.0±0.9 sec while applying 70V, and 1.9±0.9 sec for 90V. We selected 1 Hz and 70V to shorten treatment time while maximizing re-perfusion of MB for the subsequent therapy pulse. Fluorescence microscopy showed greater SPN concentration in tumor samples with longer treatment time, resulting in optimal delivery time of 100 sec. Drug delivery with optimized US parameters (Fig. 1, b), compared to the therapy using non-optimized US parameters (Fig. 1, c), showed: 1) up to 10% increase of total SPN signal, 2) up to 20% increase of SPN signal at penetration depth of 25 µm and greater from the blood vessels into the cancer tumor.

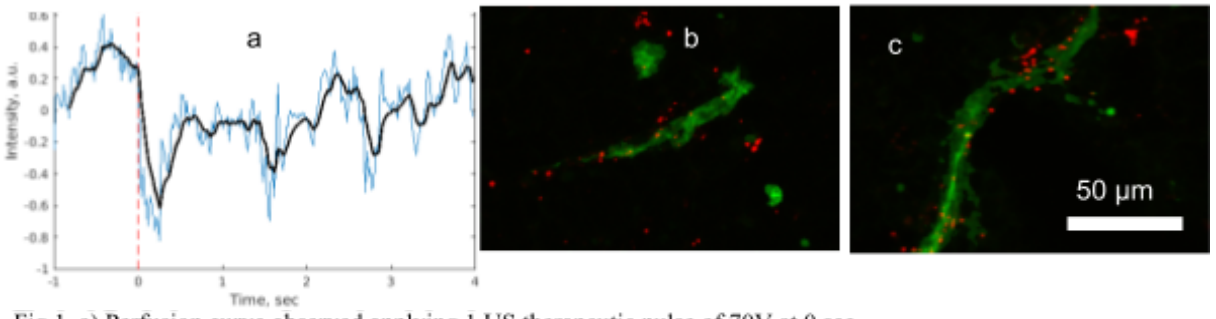

Fig 1. a) Perfusion curve observed applying 1 US therapeutic pulse of 70V at 0 sec. Immunofluorescence images of SPN model drug (red) in mice tumor with CD31 stained for endothelial marker CD31 (green): treatment using b) optimized US parameters, c) non-optimized parameters