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Quantitative Ultrasound Assessment of Ultrasound Therapy in Rodent Mammary Tumors: *In Vivo* and *Ex Vivo* Results

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Abstract— High-intensity focused ultrasound (HIFU) is a promising means of non-invasive therapy for the treatment of tumors. Monitoring and assessment challenges for HIFU therapy remain, however, and ultrasound is under investigation to accomplish non-invasive treatment feedback. Quantitative ultrasound (QUS) was investigated for acute HIFU therapy assessment as well as temperature monitoring in a rat model mammary tumor both *in vivo* and *ex vivo*. In the *in vivo* study, 40 rodent mammary tumors were exposed to focused ultrasound produced by a 1-MHz single-element transducer (f/1.1) at three spatial-peak temporal-average intensity levels (335, 360, and 502 W/cm^2). Ultrasound assessment scans were performed on each tumor before and again immediately after HIFU exposure using clinical (Ultrasonix L14/5, 3-8 MHz) and small-animal high-frequency (VisualSonics MS-200, 6-14 MHz) ultrasound systems.

For comparison purposes, twelve additional tumors were excised and scanned using a 20-MHz single element transducer before and again immediately after treatment in a 60 ºC saline bath for 30 minutes to determine both scattering and attenuation changes with thermal insult. These treated tumors were again scanned at elevated temperature (40, 45, and 50 ºC). For the excised tumors, a bandwidth of 7 to 25 MHz was used for backscatter analysis.

Backscatter coefficient (BSC) and integrated backscatter coefficient (IBSC) estimates were generated *in vivo* for each tumor, both before and after treatment. A statistically significant ($p<0.05$) difference was observed in the change in IBSC with treatment for the first exposure group (335 W/cm^2) compared to controls. In *ex vivo* tumor samples a statistically significant difference (increase) in BSC was observed between pre-treatment and post-treatment scan data. BSC was observed to decrease with increasing tumor temperature. These changes were not statistically significant, however. These studies demonstrated increases in the BSC in rodent mammary tumors with therapy in both *ex vivo*, water bath exposures and *in vivo* HIFU exposures. This work was supported by NIH Grant R01-EB008992.

Keywords: Quantiative ultrasound; Tissue characterization; HIFU;

I. Introduction

Quantitative ultrasound (QUS) tissue characterization techniques have been applied to differentiate between different types of tissues and tumors [1, 2], distinguish healthy from diseased tissues [3, 4, 5], and to quantify tissue response to therapy [6, 7]. In particular, QUS techniques have shown sensitivity to microstructural changes induced by chemotherapy and radiation. Changes in spectral features were associated with apoptosis [6, 7]. Therefore, there is a history of utilizing changes in ultrasound backscatter coefficients (BSCs) to characterize tissue states and to detect and assess therapy response.

High intensity focused ultrasound (HIFU) is an effective non-invasive cancer therapy modality which uses heat generated from absorbed ultrasound to treat tissues. HIFU therapy is currently approved for clinical use in the United States for treating uterine fibroids, and is undergoing trials for prostate cancer treatment [8, 9]. In addition, clinical trials have been conducted in Europe for HIFU treatment of breast cancer [10], liver cancer [11], and benign prostatic hyperplasia [12]. Feedback of HIFU treatment is critically important, and to date MRI has been used for temperature monitoring during HIFU treatment [13, 14]. However, MRI is an expensive modality and requires compatible ultrasound equipment. In this study we investigate a novel ultrasound-based technique, quantitative ultrasound (QUS), as a means to assess HIFU therapy and thus provide a less expensive and more portable alternative to MRI guidance.

II. Methods

A. Ex Vivo Data Acquisition and Therapy

Twelve mammary adenocarcinoma tumor (MAT) tumors were extracted immediately after euthanasia. Each tumor was trimmed to remove excess tissue and placed in a 35 ºC saline bath for scanning. Backscatter and insertion-loss scans were performed using a flat reference plate and a 20 MHz f/4 transducer excited by a Panametrics 5900 pulser-receiver

(Olympus NDT, Waltham, MA) connected to a PC A/D card with 250 MHz sampling. Transducer position was controlled using a Daedal positioning system (Daedal, Inc., Harrisburg, PA) connected to a PC running custom LabView (National Instruments, Austin, TX) software. Tumors were then removed from the saline bath at 35 ºC and placed in a saline treatment bath (held at 60 ºC) for 30 minutes. This thermal dose was chosen to provide a uniform treatment sufficient to damage the tissue as well as to avoid additional irreversible changes during scans at elevated temperature. The goal of the scans at elevated temperature was to isolate the effects of temperature from irreversible changes. Afterwards, each tumor was placed back into the saline bath held at 35 ºC and all ultrasound scans were repeated at tumor and saline bath temperatures of 35, 40, 45, and 50 ºC.

BSCs were estimated using a planar reflector reference and the method of Chen [15], and attenuation was estimated using an insertion loss technique [16]. A separate planar reference was taken at each scan bath temperature.

B. *In vivo Data Acquisition and Therapy*

Experimental procedures for this study are described elsewhere in detail [17]. Briefly, rodents with at least one 7-mm or larger tumor were anesthetized with isoflourane gas. Each tumor was scanned prior to and immediately after exposure to HIFU using both a clinical system (Sonix RP, Ultrasonix, Richmond, BC, Canada) with an L14-5/38 probe and a small-animal high-frequency (Vevo 2100, Visualsonics, Toronto, Ontario, Canada) ultrasound imaging system with an MS-200 or MS-250 probe. An average BSC curve was computed for each tumor before and again after treatment. BSCs were estimated over a 3-14 MHz frequency range using a reference phantom approach [18].

Each tumor was exposed to HIFU using an air-backed 1-MHz f/1.1 transducer connected to a power amplifier (A150 55 dB, ENI, Rochester, NY) and excited by an arbitrary waveform generator (HP 33120a, Agilent Technologies, Santa Clara, CA). Temperature increases were monitored using a needle thermocouple (HYP-1, Omega, Stamford, CT) placed at the edge of the tumors to minimize interaction with the beam.

III. RESULTS

Average *in vivo* BSC estimates for one exposure group (335 W/cm^2 I_{SPTA}) are summarized in Fig. 1. Error bars represent standard deviation and are computed with one average BSC estimate per tumor. The increase in the mean value of the BSC after HIFU treatment was significant compared to controls for this exposure group [17] using the Visualsonics data as determined by comparing the integrated BSC. The offset in the overlapping frequency band (6-8) MHz between the two array systems (Fig. 1) is likely a result of clutter caused by the strongly scattering skin, to which the more weakly focused Sonix RP system was more sensitive.

Comparable increases can be observed for *ex vivo* BSC estimates (Fig. 2). *In vivo* estimates used a nominal attenuation value of 0.7 dB/cm/MHz for all tumors, while *ex vivo* tumor estimates were compensated using average attenuation coefficient estimates from insertion loss measurements of the same tumors (table 1). Attenuation coefficients increased substantially with bath treatment, which is consistent with protein denaturation and the response observed in many other studies with different tissues [19, 20]. Comparing the BSC data in Fig. 2 using multivariate analysis of variance (MANOVA), a statistically significant difference ($p<0.01$) was found between the pre and post-treatment BSC curves.

BSCs of the treated tumors were observed to decrease with increasing temperature (Fig. 3). This behavior is comparable to temperature-dependent BSC estimates in liver and cell pellets [21], though these estimates were generated under slightly different experimental conditions. Statistical analysis (MANOVA) did not reveal a significant difference between these four BSC curves examined together.

TTC staining of *ex vivo* treated MAT tumors revealed a uniform and substantial loss of cell viability after exposure to the therapy bath for 30 minutes, but not after the first (untreated) scan before placement in the treatment bath (Fig. 4). TTC stains were also used to quantify treatment response in the *in vivo* experiment and reduced tumor viability was similarly produced, though not in the uniform fashion observed *ex vivo* [17]. In both cases, tumor necrosis was confounding factor in assessing viability with this stain.

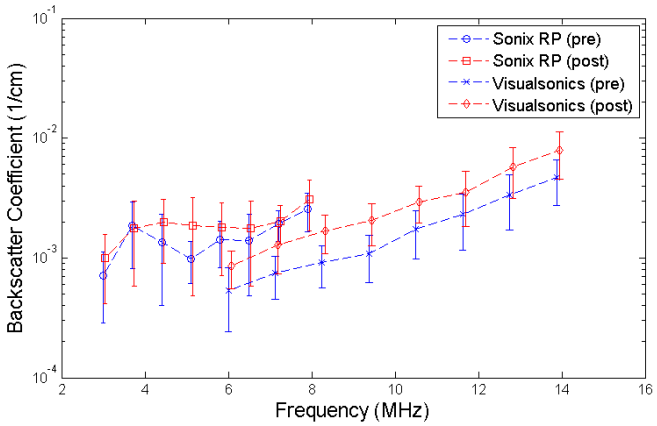

Figure 1 – Average BSC estimates for in vivo tumors (Sonix RP and Visualsonics systems)

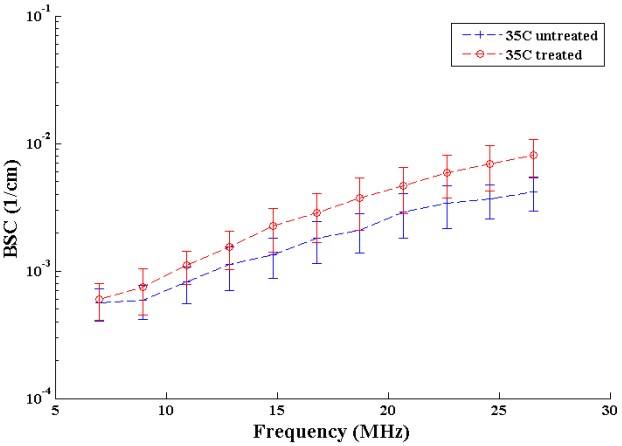

Figure 2 – Average BSC estimates for *ex vivo* tumors before and after treatment (20 MHz single-element transducer, 35 °C bath temperature)

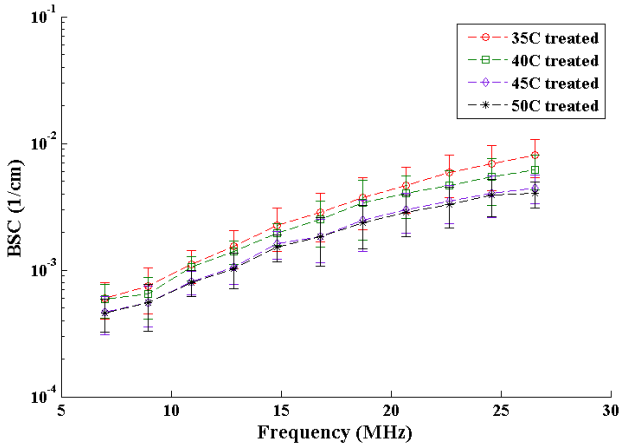

Figure 3 – Average BSC estimates for treated *ex vivo* tumors at four tumor temperatures (20 MHz single-element transducer)

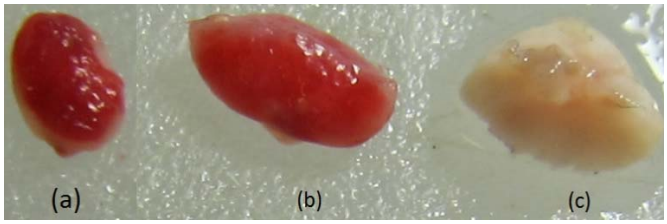

Figure 4 – TTC staining (*ex vivo* scan tumors): (a) after extraction, (b) before therapy bath exposure, and (c) after therapy bath exposure. Viable regions uptake stain (pink).

TABLE I. POWER LAW ATTENUATION COEFFICIENTS FOR ATTENUATION COMPENSATION

	Tumor attenuation compensation	
	alpha	***N***
In vivo	0.7	1
Ex vivo (pre 35ºC)	0.41	1.134
Ex vivo (post, 35 ºC)	0.58	1.124
Ex vivo (post, 40 ºC)	0.55	1.119
Ex vivo (post, 45 ºC)	0.43	1.169
Ex vivo (post, 50 ºC)	0.39	1.182

IV. CONCLUSIONS

Increases in ultrasound BSCs have been associated with therapeutic response in tumors. When investigating HIFU therapy in particular, *in vivo* BSCs increased in 2 of 3 exposures from 6-14 MHz [17]. An *ex vivo* experiment from 7 – 27 MHz was conducted to isolate the role of thermal therapeutic effects from potential mechanical effects (i.e., boiling and cavitation) and to accurately estimate attenuation coefficients in normal and thermally treated MAT tumors. An increase in the BSC was observed with therapy, and MANOVA analysis revealed that this increase was statistically significant.

Estimates of ultrasonic properties from measurements taken at four different temperatures (up to 50 ºC) from MAT tumors treated in the same fashion were compared. Although a trend of decreasing BSC estimates was observed, statistical significance was not achieved for the sample size examined.

While increases in BSC *in vivo* were initially hypothesized to be the result of mechanical effects such as boiling or cavitation, increases in BSC were measured *ex vivo* in the absence of these effects. Thus, while mechanical effects may also contribute to BSC increases, the *ex vivo* scan results suggest that thermal effects may also generate BSC increases in tumor tissues at higher frequencies. Attenuation estimates increased with treatment (Table 1), which was also observed in rodent liver exposed *ex vivo* to a thermal bath, while increases in BSC (Fig. 2) were not observed in the same rodent liver [20].

Combined, these studies suggest that increases in backscatter coefficients may be an important ultrasonic signature of HIFU therapy in tumors and an important means to detect when therapy has successfully occurred. These increases may disappear at elevated temperature and may be tissue dependent, however.

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