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14. ABSTRACT

Neoadjuvant chemotherapy is currently the standard of care for locally advanced breast cancer (LABC). Monitoring tumor response is advantageous for patients. This project aims at establishing noninvasive monitoring of neoadjuvant chemotherapy in the breast using subharmonic aided pressure estimation (SHAPE) to estimate the interstitial fluid pressure (IFP) in LABC. New in vitro experiments with the ultrasound contrast agent Definity have showed an inverse linear relationship between the change in subharmonic amplitude and hydrostatic pressure ($r2 = 0.63 - 0.95$, $p < 0.01$) over the pressure range associated with breast tumors (0 – 50 mmHg). Moreover, software for analyzing RF data from a Sonix RP scanner to produce SHAPE pressure estimates has been successfully optimized and published. In vivoproof of concept for SHAPE as a noninvasive monitor of IFP ($r2 = 0.67 - 0.96$, $p < 0.01$) has been provided based on a swine model. The work on in vivo SHAPE measurements of IFPwas selected for the final of the American Institute of Ultrasound in Medicine (AIUM) 2011 Young Investigator Award (for V. G. Halldorsdottir) and was awarded FIRST place out of 90 abstracts submitted for this competition.

15. SUBJECT TERMS
Breast Cancer, Ultrasound Imaging, Ultrasound Contrast Agent, Pressure Estimation, Neoadjuvant chemotherapy

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4 INTRODUCTION

In the United States, close to 5 – 20 % of newly diagnosed breast cancer and 10 – 30% of all primary breast cancer is diagnosed as locally advanced breast cancer (LABC) [1, 2]. Neoadjuvant chemotherapy (systemic preoperative chemotherapy) is currently the standard of care for LABC [3, 4]. When compared with adjuvant chemotherapy (postoperative therapy), neoadjuvant chemotherapy yields similar results for both overall survival (70% for both) and disease-free survival (53% adjuvant, 55% neoadjuvant) [5]. Thus, the postponement of surgery does not affect the outcome of the treatment [5, 6]. In addition, neoadjuvant chemotherapy offers considerable benefits to the patient as the treatment can shrink the tumor and even in some cases offer complete pathologic response [3, 7]. This reduction in tumor size increases the possibility of breast conservation [3, 5-7]. Maximizing the conservation of breast tissue can be of great personal importance for the self-esteem and quality of living of the patient [6]. Neoadjuvant chemotherapy can also offer an early indication of the patient's response to chemotherapy. Consequently, monitoring tumor response to neoadjuvant therapy gives the possibility of adjusting the treatment if the patient is responding poorly or not at all resulting in substantial advantages for the patient [3, 6]. This project aims at establishing noninvasive monitoring of neoadjuvant chemotherapy in the breast using subharmonic aided pressure estimation (SHAPE; U.S. Patent 6,302,845).

Generally interstitial fluid pressure (IFP) is 10-30 mmHg higher in cancerous tissue than in normal tissue although values of up to 60 mmHg have been recorded [8, 9]. Similarly, IFP in breast cancer tumors has been shown to be higher than that of surrounding breast tissue [9]. This increase is believed to be due to vascularity, fibrosis and difference in the interstitial matrix in tumors and it can result in poor transport of therapeutic drugs to tumors [8]. Taghian et al. used a wick-in-needle technique to monitor the IFP of breast cancer before and after neoadjuvant chemotherapy with two drugs used consecutively [10]. When used as a first drug Paclitaxel decreased the IFP by 36% ($p=0.02$) whereas with Doxorubicin as a first drug there was only 8% reduction ($p=0.41$). As this was a hypothesis-generating study they did not show any outcome related to the relationship between IFP and therapy response [10]. However, the level of IFP has been shown to predict disease free survival for cervix cancer (34% disease free survival (DFS) if IFP > 19 mmHg, 68% DFS if IFP < 19 mmHg ($p = 0.002$)) [11]. Thus, the level of interstitial fluid pressure (IFP) in breast cancer tumors could potentially be used to monitor the response to neoadjuvant chemotherapy.

Contrast agents have been used for two decades to improve visualization in ultrasound (US) imaging as they enhance the difference in reflectivity between tissues [12]. Because of the difference in compressibility between the medium and the microbubble any changes in pressure induce changes in the size of the microbubble [13]. This in turn affects the reflectivity and resonant frequency of the bubble [13, 14]. In subharmonic imaging (SHI) pulses are transmitted at a frequency f_0 and the echoes are received at half that frequency $f_0/2$. SHI has been showed to be a feasible option for contrast enhanced imaging due to subharmonic generation by contrast agents and limited subharmonic generation in tissues [15]. Our group came up with a novel technique, SHAPE, utilizing

microbubbles and the subharmonic amplitude of the scattered signal [13]. We showed that there is a linear relationship between the hydrostatic pressure and the subharmonic amplitude. We propose the use of SHAPE to monitor treatment response by noninvasively measuring the IFP in breast tumors. This offers several benefits to the patient. As opposed to the wick-in-needle technique SHAPE is noninvasive and does not inflict pain. Furthermore, it allows for an early indication of responders vs. non-responders and thereby makes adjustments to therapy easier. Moreover, SHAPE has been shown to have a favorable signal-to-noise ratio so the subharmonic amplitude is not affected by background noise [13].

The optimal contrast agent and acoustic parameters for SHAPE will be established using *in vitro* pulse-echo measurements. The SHAPE algorithm will then be designed and implemented on a commercial, state-of-the-art US scanner for *in vivo* IFP measurements. A similar algorithm has already been set up for cardiac SHAPE and thus only a few adjustments need to be made to implement SHAPE for breast tumors making this very cost-effective. The *in vivo* experiments will be twofold. First, athymic, nude, female rats will be implanted with SKBR3, MCF-7 or BT474 human breast cancer cells and SHAPE used to measure IFP and calibrated by comparing the SHAPE results to IFP measurements obtained with an invasive, intra-compartmental pressure monitor as the gold standard. After calibration, human xenograft breast tumors in athymic, nude, female rats will be used to evaluate the ability of SHAPE to track changes in IFP by studying before and after administration of a chemotherapy agent (paclitaxel).

Our group has proposed that SHAPE and contrast enhanced US imaging can be used to measure the IFP in LABC tumors, thus, making it possible to noninvasively monitor the tumor response to neoadjuvant chemotherapy. This method would be a considerable improvement from the wick-in-needle technique currently used for IFP measurements in LABC and allow for individualized treatments options.

5 BODY

The hypothesis of this project is that IFP in breast tumors can be measured noninvasively using SHAPE and contrast enhanced US thus improving the monitoring of neoadjuvant chemotherapy. To investigate this prospect, *in vitro* pulse-echo experiments will be conducted to investigate this prospect and find the optimal contrast agent for SHAPE. These results will then be used to implement SHAPE on a commercial scanner. The scanner will be used for *in vivo* studies on 201 rats with tumor xenografts in order to calibrate and evaluate SHAPE's ability to monitor response to neoadjuvant chemotherapy. The specific tasks of the project (as presented in the original Statement of Work) can be found in Appendix I.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

5.1 Methods

In vitro experiments

Last year the static pressure measurements were repeated using small (10 ml) OptiCell chambers (Nunc, Rochester, NY). However, after some extensive testing, we discovered that standing waves markedly influenced results and made measurements impossible to reproduce. Hence, our use of the OptiCell setup had to be abandoned and a completely new tank (with extra acoustic absorbers incorporated to minimize reverberations) had to be designed.

The new water tank was constructed and the pressure inside the tank was monitored by a pressure gauge (OMEGA Engineering, Stamford, CT; Fig 1). A state-of-the-art commercial scanner Sonix RP (Ultrasonix Medical Corporation, Richmond, BC, Canada) with a high frequency linear array (L14-5/38) was used to acquire radio-frequency (RF) data at the focal zone depth (4.25 cm) at a 9 Hz framerate following injection of the contrast agent Definity (Lantheus Medical Imaging, N Billerica, MA) in a 0.2 ml/l dose. Two different transmit frequencies of 6.7 and 10 MHz were considered. Contrast echoes were received at half the transmit frequencies (i.e., 3.35 and 5.0 MHz). The acoustic output power was varied from 0 to -20 dB (in 2 dB steps; 0.24-2.05 MPa) for a 0 mmHg hydrostatic pressure in order to establish the optimal sensitivity for SHAPE. Then the chamber pressure was varied from 0 to 50 mmHg to simulate IFP in tumors and acoustic pressure varied from -16 to -4 dB (in 4 dB steps). After RF data acquisition, files were transferred to a PC and the amplitude of the subharmonic signal component was extracted using MATLAB (Mathworks, Natick, MA) as shown in Figure 2. Three measurements were acquired at each setting and linear regression analysis used to determine the relationship between hydrostatic pressure and change in subharmonic amplitude. All statistical analyses were conducted using Stata 9.0 (Stata Corporation, College Station, TX).

Figure 1. *The re-designed water tank scanned by the L14-5 transducer. Notice the digital pressure gauge on the top of the tank.*

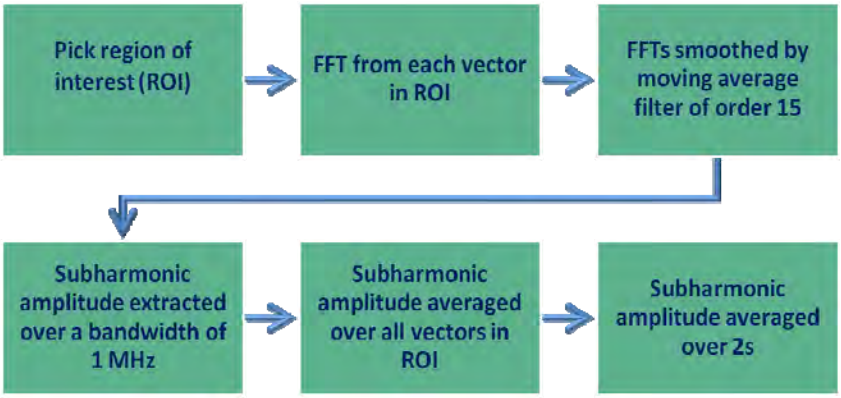

Figure 2. *Block diagram of the off-line processing performed on the RF data.*

Moreover, a novel, simulation model of the dynamics of an encapsulated microbubble contrast agent, developed as part of a previous DOD supported project [14], was modified in order to account for ambient pressure variations and different shell parameters to establish the optimal contrast microbubble for SHAPE. A nonlinear extension of the original viscoelastic model was pursued by considering a quadratic elasticity model where the interfacial elasticity vary linearly with area fraction as well as an exponent.

In vivo experiments

Our group has worked in partnership with Ultrasonix Medical Corporation to implement SHAPE for cardiac use on a state-of-the-art commercial scanner Sonix RP (Ultrasonix Medical Corporation, Richmond, BC, Canada) with a phased array (PA4-2). Several experiments have been carried out in canines to investigate cardiac SHAPE supported by funding from the AHA. RF data from these experiments was analyzed off-line using Matlab. The software developed by our group for this analysis is not site-specific and will also be used to analyze the data we will acquire from *in vivo* breast SHAPE in rats as part of this project. This year several manuscripts based on the optimization of this software were submitted for publication.

Finally, an opportunity to provide a proof-of-concept of the use of SHAPE for estimating IFP and test the invasive (needle based) Stryker pressure monitoring system (the reference standard) presented itself. As part of an ongoing NIH study, a unique, naturally occurring tumor model, the Sinclair swine with melanoma, was being studied. We obtained IFP measurements from the tumors and surrounding tissue using the Stryker needle based system, which provided us with a chance to assess the dependence of this technique on the angle between the needle and the tissue. Moreover, subharmonic signals were acquired during an infusion of Definity (6.25 ml/l/min) with the Sonix RP and a linear array. Data were obtained at 6.7 and 10 MHz (i.e., subharmonic frequencies of 3.75 and 5.0 MHz, respectively) with acoustic outputs of -4, -8 and -12 dB and analyzed further during the last year. Five (5) swine were studied (one melanoma per swine) at no cost to this project.

5.2 Results and Discussion

In vitro experiments

Subharmonic signal generation as a function of incident acoustic pressure can be characterized into 3 stages – occurrence, growth and saturation (Fig. 3). In the growth stage, the subharmonic amplitude decreased linearly with an increase in ambient pressure similar to our previous results [13]. Thus, subharmonic signals from microbubbles in the growth stage can be calibrated to provide the ambient overpressures with optimal sensitivity. Part of this work has been published [15].

Over the pressure range of 0 – 50 mmHg (simulating the IFP in breast tumors) *in vitro*, pressures were inversely related to the change in subharmonic amplitude ($r2 \geq 0.63$; $p < 0.05$; Figure 4). Receiving at 3.35 MHz is at the lower end of the probe bandwidth, which may explain why results at 5.0 MHz were almost always better. The maximum decrease obtained with Definity microbubbles over 50 mmHg was 11.36 dB ($r^2 = 0.95$; $p < 0.01$) at 10 MHz and -8 dB power (Figure 5). This is consistent with previous results reported by our group [13] and these efforts represent the fulfillment of tasks 1a, 1d and 1f in the original Statement of Work (SOW).

The previously developed simulation model [14] was modified to better account for the experimentally observed changes in subharmonic signal amplitudes as a function of hydrostatic pressures. The model showed that the determining parameter of subharmonic response is the ratio of the excitation to the resonance frequency. For different acoustic excitation pressure levels, changing ambient pressure can either increase or decrease the subharmonic response depending on this ratio. For some range of parameters, the variation is far more complicated. This behavior is clearly at odds with the experimental observations mentioned above and is currently not well understood. This investigation has been published [16, 17]. This constitutes the continuation of tasks 1b, 1c and 1e.

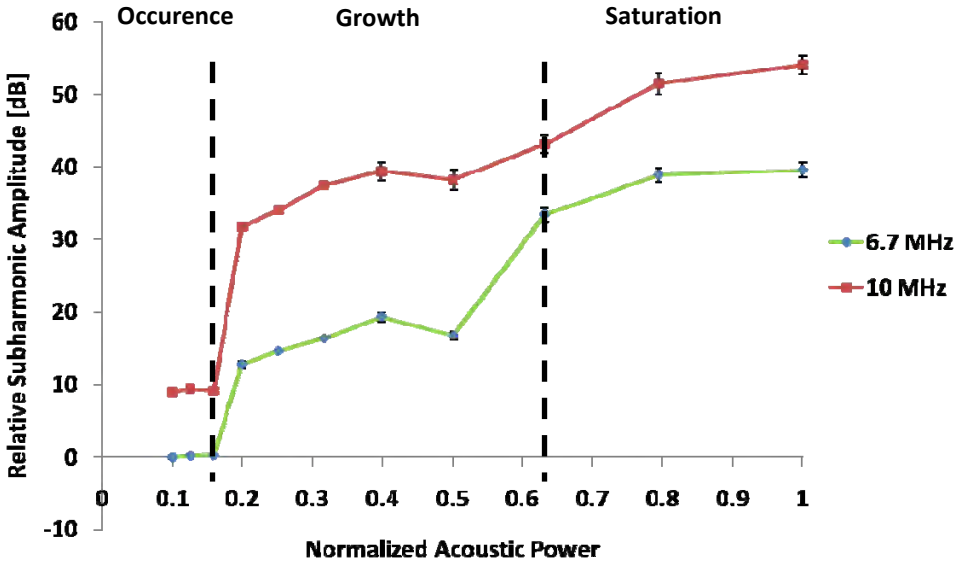

Figure 3. *Subharmonic response to changes in acoustic power with the occurrence, growth and saturation phases clearly seen.*

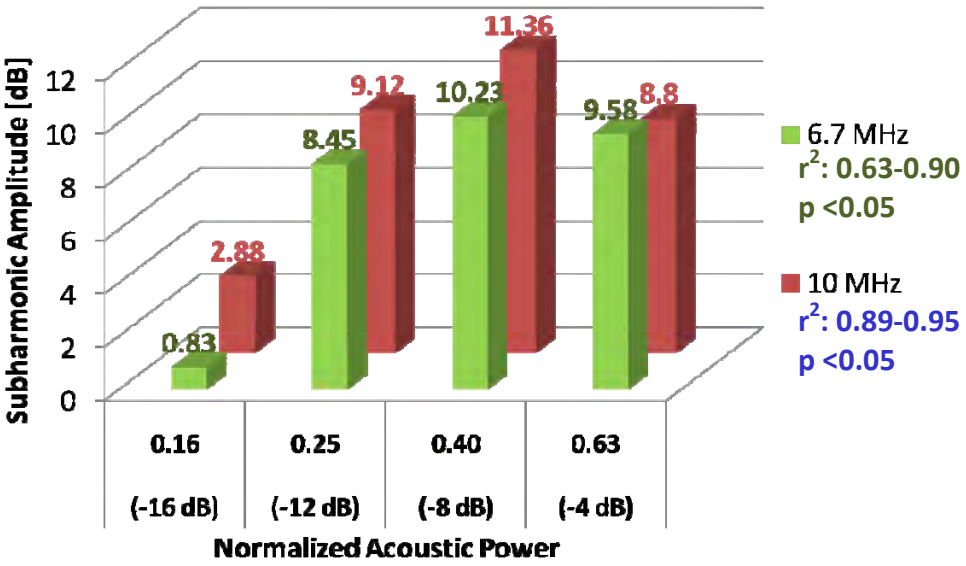

Figure 4. *Maximum decrease in subharmonic signal amplitude for Definity as a function of frequency and acoustic power (N = 3).*

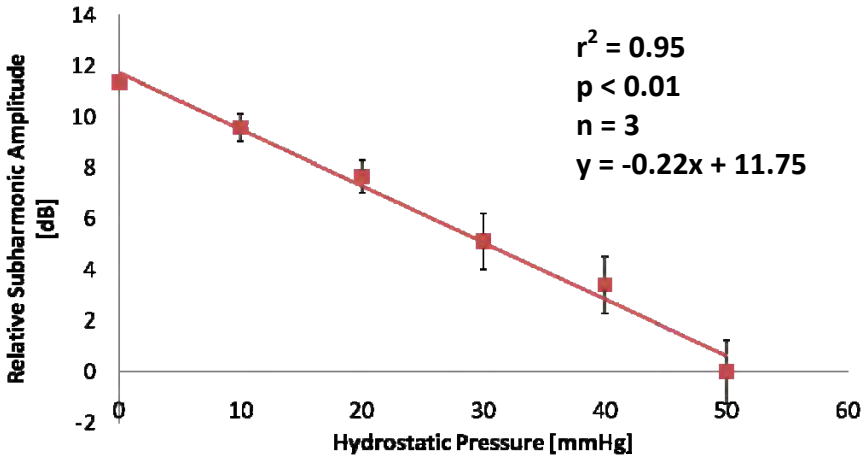

Figure 5. *Inverse linear relationship between pressure and subharmonic signal amplitude for Definity at 10 MHz and -8 dB acoustic output power (N = 3).*

In vivo experiments

Software to analyze RF data from the Sonix RP scanner has been improved and the best method to extract the subharmonic signal components from the frequency spectrum has been established, as described in last year's report. This work has described in four (4) manuscripts out of which one has been published, two are "in press" and the last paper is still under review [18-21]. Moreover, a patent application has been submitted to the U.S Patent Office based on these development efforts [22]. This effort represents the conclusion of task 2b.

A unique opportunity to test the Stryker pressure monitoring system and provide proof-of-concept of the use of SHAPE for estimating IFP was pursued in the Sinclair swine melanoma model. Five (5) swine were studied, but one of the swine was eliminated from the study, due to technical difficulties. The difference between the subharmonics and the IFP in tumors and their surrounding tissues was most linear for 10 MHz transmission ($r^2 \geq 0.67$; $p < 0.05$; Fig. 6) [23]. The difference between tissue and tumor IFP is rather large and may be caused by the sensitivity of the Stryker system to the angle between the needle and the tissue being studied (this issue is currently being investigated further). The slope from 3 out of 4 animals was very similar to each other (-0.19 ± 0.07) and to the *in* vitro slope (-0.22), while the last swine (i.e., swine 1) showed a large spread within the normal tissue IFP. In conclusion, these results provide proof of concept for the feasibility of using SHAPE as a noninvasive monitor of IFP.

Finally, the work on *in vivo* SHAPE measurements of IFP [23] was selected for the final of the American Institute of Ultrasound in Medicine (AIUM) 2011 Young Investigator Award (for V. G. Halldorsdottir) and was awarded FIRST place out of 90 abstracts submitted for this competition.

Regulatory Review

The animal experiments scheduled for the final year of this project have received approval from the university's Institutional Animal Use and Care committee (IACUC) in Year 1 of this project. However, this year we prepared and submitted an expanded protocol to Lantheus Medical Imaging in order to obtain support for this project in the form of the ultrasound contrast agent Definity. The research committee at Lantheus recently approved our project for support and committed to delivering 230 vials of Definity (at no cost to this project) for the *in vivo* experiments. This effort represents the conclusion of task 2d.

6 KEY RESEARCH ACCOMPLISHMENTS

- SHAPE experiments were conducted *in vitro* in a new, improved water-tank.
- The maximum decrease obtained with Definity over 50 mmHg was 11.36 dB (r^2=0.95; p<0.01) at 10 MHz and -8 dB power.
- Software for processing of *in vivo* SHAPE data has been further optimized.
- *In vivo* proof of concept for SHAPE as a noninvasive monitor of IFP has been provided ($r^2 = 0.67 – 0.96$, $p < 0.01$).
- The L9-4 linear array has been selected for the *in vivo* studies and scanner design requirements have been developed.
- Lantheus Medical decided to support this project with 230 vials of Definity for use in the *in vivo* experiments.

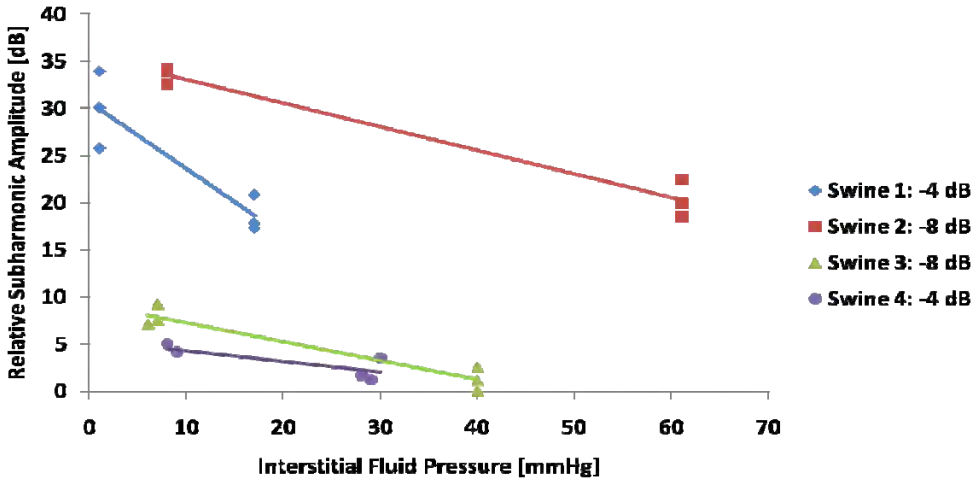

Figure 6. *In vivo measurements showing SHAPE results compared to the pressure monitor. The relative difference between tissue and tumor IFP is clearly captured by SHAPE.*

7 REPORTABLE OUTCOMES

Publications

J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, J. B. Liu, F. Lin, J. H. Zhou, H. K. Wang, K. Thomenius, F Forsberg. In vivo subharmonic pressure estimation of portal hypertension in canines. *Proc IEEE US Symp*, pp. 778-781, 2010.

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F. Forsberg, J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, J. S. Raichlen, J. B. Liu, C. Miller, J. M. Gonzalez, M. E. McDonald, D. A. Merton, D. Brown, V. Navarro. On the utility of subharmonic contrast microbubble signals. *Ultrasonic Imaging*, vol. 33, pp. 65-66, 2011.

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J. R. Eisenbrey, J. K. Dave, V. G. Halldorsdottir, S. Park, S. Dianis, D. A. Merton, P. Machado, J. B. Liu, J. M. Gonzalez, C. Miller, K.E. Thomenius, D. B. Brown, V. Navarro, F. Forsberg. Dual grayscale and subharmonic ultrasound imaging on modified ultrasound scanner in 2 and 3D. Accepted for publication in *Proc IEEE US Symp*, 2011.

J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, J. B. Liu, M. E. McDonald, K. Dickie, C. Leung, F. Forsberg. Noninvasive estimation of dynamic pressures in vitro and in vivo using the subharmonic response from microbubbles. Accepted for publication in *Proc IEEE US Symp*, 2011.

F. Forsberg, J. R. Eisenbrey, J. K. Dave, V. G. Halldorsdottir, J. B. Liu, J. M. Gonzalez, C. Miller, P. Machado, D. A. Merton, S. Park, S. Dianis, C. L. Chalek, K.E. Thomenius, D. B. Brown, V. Navarro. Dual grayscale and subharmonic US imaging on a modified commercial scanner. Accepted for publication in *Prog RSNA*, 2011.

V. G. Halldorsdottir, J. Eisenbrey, J. K. Dave, F. Forsberg, P. Machado, B. C. Cavanaugh, D. A. Merton, J. B. Liu. Subharmonic aided pressure estimation for monitoring interstitial fluid pressure in swine melanomas: initial in vitro and in vivo results. Accepted for publication in *Prog RSNA*, 2011.

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J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, J. S. Raichlen, J. B. Liu, M. E. McDonald, K. Dickie, S. Wang, C. Leung, F. Forsberg. Noninvasive left ventricular pressure estimation using subharmonic emissions from microbubbles - an in vivo pilot study. *JACC Cardiovasc Imaging*, vol. 4, 2011. In press.

J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey, F. Forsberg. Processing of subharmonic signals from ultrasound contrast agents to determine ambient pressures. Submitted to *Med Phys*, September, 2011

Presentations

September 30 - October 1, 2010	25th Annual Advances in Contrast Ultrasound & ICUS Bubble Course 2010, Chicago, IL, USA. • In vivo cardiac subharmonic pressure estimation.
October 11 - 14, 2010	IEEE 2010 International Ultrasonics Symposium, San Diego, CA, USA. • In vivo subharmonic pressure estimation of portal hypertension in canines.
November 21–23, 2010	63rd Annual Meeting of the American Physical Society, Division of Fluid Dynamics Long Beach, CA, USA. • Strain-softening elasticity model of the encapsulation of an ultrasound contrast microbubble. • Subharmonic response from ultrasound contrast microbubbles for noninvasive blood pressure estimation.
April 14 - 17, 2011	The 56th Annual Convention of the American Institute of Ultrasound in Medicine, New York City, NY, USA.

- Subharmonic aided pressure estimation for monitoring interstitial fluid pressure in tumors: *in vitro* and *in vivo* proof of concept.
- Subharmonic signals for noninvasive cardiac pressure estimation – initial *in vivo* experience.

May 23-27, 2011 — Acostical Society of America meeting, Seattle, WA, USA.
- Subharmonic response from ultrasound contrast microbubbles for noninvasive blood pressure estimation

June 13 – 15, 2011 — Thirty-Sixth International Symp. on Ultrasonic Imaging and Tissue Characterization, Washington DC, USA.
- On the utility of subharmonic contrast microbubble signals.
- Dynamics of contrast microbubbles and their subharmonic response for noninvasive blood pressure estimation

August 2 – 5, 2011 — Era of Hope, Dept of Defense Breast Cancer Research Meeting, Orlando, FL, USA.
- Subharmonic aided pressure estimation for monitoring neoadjuvant chemotherapy of locally advanced breast cancer (poster).

V. G. Halldorsdottir was selected as a finalist for the AIUM 2011 Young Investigator Award based on her work in the abstract labled * above. The competition was judged at the Annual Conference of the AIUM (in April 2011 in New York City, NY) and was awarded FIRST place out of 90 abstracts submitted for this competition.

Patents

F. Forsberg, J. K. Dave, V. G. Halldorsdottir, J. R. Eisenbrey. Methods for improved selection, processing and display of subharmonic microbubble signals as pressure estimates. U.S. Patent pending 61/498,278, June, 2011.

8 CONCLUSIONS

To date we have investigated six US contrast agents for use in SHAPE and shown that Definity (Lantheus Medical Imaging, N Billerica, MA) has an inverse linear relationship between the change in subharmonic amplitude and hydrostatic pressure ($r^2 = 0.63 – 0.95$, $p < 0.01$) over the pressure range associated with breast tumors (0 – 50 mmHg) when measured *in vitro* employing the new water-tank (with extra acoustic absorbers incorporated). This work has been partially published [15].

Our attempts to design a realistic simulation model accounting for the experimental results have been mixed and further work is ongoing. These efforts have been published [16, 17]. Software for analyzing RF data from the Sonix RP scanner to produce SHAPE

pressure estimates has been successfully optimized and several publications have been published or are in press or submitted for review [18-21].

Finally, *in vivo* proof-of-concept for SHAPE as a noninvasive monitor of IFP has been provided in 5 swine with naturally occurring melanoma. SHAPE showed excellent correlation with IFP values obtained in normal tissues and in the tumor using intra-compartmental pressure measurements with the wick-in-a-needle technique ($r^2 = 0.67 – 0.96$, $p < 0.01$). The work on *in vivo* SHAPE measurements of IFP was selected for the final of the American Institute of Ultrasound in Medicine (AIUM) 2011 Young Investigator Award (for V. G. Halldorsdottir) and was awarded FIRST place out of 90 abstracts submitted for this competition [23].

Given the results above and the fact that our group has published twenty-five (25) abstracts at national and international conferences as well as seven (7) peer-reviewed papers and one (1) patent application in the almost 3 years the original BRCP award have been underway, we have submitted an IDEA Expansion proposal to the BRCP. This proposal builds on the work achieved in our prior BCRP awards by performing the all important translation of these methods towards clinical feasibility by conducting a first-in-humans clinical trial of 3D SHAPE in women undergoing neoadjuvant chemotherapy.

In summary, task 1 has been almost completely completed while task 2 is ongoing. However, due to the delay caused by the *in vitro* experiments the project has been granted a one year no cost extension during which task 3 will be completed.

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Appendix I

The Statement of Work from the original proposal:

Objective 1
Task 1: Computer modeling and *in vitro* experiments (months 1 - 6)

a. Construct an *in vitro* experimental pulse-echo system for investigating the effect of hydrostatic pressure variations on contrast microbubbles and measuring the resulting changes in backscattering (Month 1).
b. Design and modify numerical codes for a theoretical model describing the dynamics of contrast microbubbles under different pressure conditions (Months 1 - 3).
c. Calculate the behavior of individual contrast microbubble and the collective behavior of contrast microbubble populations (Months 3 - 6).
d. Measure changes in backscattered fundamental, second and subharmonic signals for different contrast agents as a function of pressure (Months 2 - 6).
e. Predict optimal contrast agents for SHAPE according to the numerical simulations (Month 6).
f. Select optimal contrast agent(s) for SHI and SHAPE. The selection will mainly be based on experimental measurements (Month 6).

Objectives 2 - 3
Task 2: Design and implementation of SHAPE on a commercial US scanner (months 7 - 12)

a. Optimize SHI and SHAPE, based on *in vitro* measurements and simulations using the actual parameters of the designated transducers (Months 7 – 8).
b. Modify a state-of-the-art US imaging system (the Sonix RP) to incorporate the SHI contrast imaging modality and to perform SHAPE (Months 8 - 10).
c. Evaluate the new imaging modality and SHAPE in an *in vitro* phantom using the modified US scanner (Months 11 - 12).
d. Prepare regulatory review and obtain approval for animal studies (Months 9 - 12).

Objectives 3 - 5
Task 3: Animal experiments, data collection and analysis (months 13- 36)

a. Create and grow breast tumors by implanting one of three human breast cancer cell lines (SKBR3, BT474 or MCF-7) into the mammary fat pad of athymic, nude rats (Months 13 - 34).
b. Calibrate *in vivo* SHAPE results based on IFP measurements obtained with the intra-compartmental pressure monitor in 21 nude rats. Three groups (one per cell line) of 7 rats with breast tumors implanted will be studied (months 14 - 16).

c. Produce and evaluate the ability of SHI to depict normal vascularity as well as breast tumor angiogenesis in human xenografts implanted in nude rats compared to CD31 stained specimens (Months 17 - 34).
d. Validate the clinical potential of SHAPE as a therapy monitoring tool by studying 180 human xenograft breast tumors in nude rats (42 normal rats and 138 after administration of a chemotherapy agent paclitaxel) and comparing results to intra-compartmental pressure measurements (months 17 - 34).
e. Perform statistical analyses and write final report (months 34 - 36).