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14. ABSTRACT

Chemotherapy is becoming more important in breast cancer treatment. It offers a perfect opportunity to evaluate the utility of newer targeted drugs. Despite the development of new techniques to characterize the biologic features of breast tumors, the factors influencing the quality of response to therapy remain obscure. One factor that may influence response to systemic chemotherapy is tumor perfusion. Tumors with relatively poor perfusion may receive inadequate delivery of systemic therapy. This lack of blood flow to the tumor may be a factor in poor response to intravenous chemotherapy. Furthermore, under perfused tumors may be hypoxic. Hypoxia has been implicated in the induction of biologic features associated with aggressive behavior and poor response to various forms of chemotherapy. Our hypotheses were a) Tumor blood vessels were leaky and therefore acoustic vibration can be used to modulate the leaky vessels and induce oxygenation changes and improve tumor oxygenation; and b)The oxygenation changes can be detected by optical measurements. Preliminary studies with 5 tumor-bearing rats demonstrated that ultrasonic vibrations could either generate significant effects (early-stage tumors) on optical measurements or no effects on optical measurements (late-stage tumors).

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Table of Contents

Introduction:

Annually, more than 200,000 American women are diagnosed with breast cancer and nearly 40,000 die from the disease. It is the second leading cause of death among American women. The treatment of breast cancer includes surgery, radiation, chemotherapy, and hormonal modification. Despite the development of new techniques to characterize the biologic features of breast tumors, the factors influencing the quality of response to therapy remain obscure. Biological makers have shown an ability to predict breast cancer response to only particular forms of therapy [1-2]. One factor that may influence response to systemic chemotherapy is tumor perfusion [3-4]. Tumors with relatively poor perfusion may receive inadequate delivery of systemic therapy. This lack of blood flow to the tumor may be a factor in poor response to intravenous chemotherapy [5]. Furthermore, under-perfused tumors may be hypoxic [6-7]. Hypoxia has been implicated in the induction of biologic features associated with aggressive behavior and poor response to various forms of chemotherapy [4]. A recent publication in Nature Medicine using the angiogenesis inhibitor bevacizumab in patients with rectal cancer, has shown this therapy to be associated with improved oxygenation and reduced blood vessel permeability within the tumor [8]. This may result in improved delivery of chemotherapy to the tumor and reduction in the potential of metastasis.

Body:

Our hypothesis was

a) Tumor blood vessels were leaky and therefore acoustic vibration can be used to modulate the leaky vessels and induce oxygenation changes and improve tumor oxygenation.

The oxygenation changes can be detected by optical measurements

The Proposed Statement of Work were

Task 1: Monitoring cancer oxygenation changes induced by ultrasound with NIR dual wavelength system (months 1 to 12)

a. Calibration and testing of the existing NIR imager for oxyHb and deoxyHb measurements.
b. Study imaging algorithms for oxyHb and deoxyHb calculation.

Task 2: Optimizing ultrasound radiation parameters toward maximizing primary radiation force (months 3 to 24)

a. Optimizing ultrasound system parameters such as pulse duration, radiation pressure, and pulse repetition frequency, toward maximizing induced oxygenation changes.
b. Instrumentation and testing of the ultrasound system.

Task 3: Conducting animal experiments with rat tumor models to assess the oxygen diffusion enhanced by ultrasound (months 4:36).

a. Animal experiments
b. Dada analysis
c. Instrumentation improvement

RESULTS OF TASK 1:

MULTI-CHANNEL THREE-WAVELENGTH NIR IMAGER

We have developed a NIR diffusive optical tomography system for animal and human subject studies. The existing NIR imager was equipped with 12 dual wavelength (780 nm and 830 nm) laser diode source pairs and 8 PMT detectors. The analysis indicated that wavelengths in the range of 660 nm to 700 nm were needed for improving the oxygen saturation estimate. Some features of the NIR imager developed include a three-wavelength excitation capability, fast optical switching in 9 transmission channels, and 8 high-dynamic range avalanche photodiode (APD) detectors. In the transmission part (see Fig. 1(a)), pigtailed laser diodes at 660, 780,

and 830 nm were used as light sources and were amplitude modulated at 140 MHz. Compared with our existing NIR imager, the wavelength excitation at 660nm allowed improved background estimation of oxygen saturation. One 4X1 and one 1X9 optical switches were combined to form a 4X9 switch, which distributed the output of one wavelength to one of nine source fibers. A total of ten parallel detectors were receiving signals from a sample simultaneously. The short switching time (about 3 ms) kept the data acquisition within 1 second for a complete scan. This made it possible to monitor dynamic physiological changes such as oxygen diffusion or perform near real time tomographic imaging. The system was well-calibrated using phantoms. Details of this system used for animal studies can be found from Ref. 9.

RESULTS OF TASK 2:

128-CHANNEL ULTRASOUND PULSER

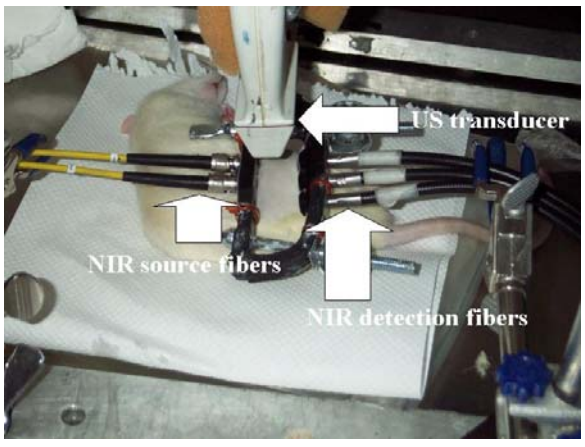
In this study, we used a 1-D existing ultrasound transducer of 196-elements with a center frequency of 7.5 MHz. The middle 128 elements were connected to an in-house designed 128-pulser ultrasound system instrumented by us. The pulser can provide pulses of a variable high-voltage up to 200 Volts peak-to-peak and pulse repetition frequency up to 2KHz. A control program was developed in LabView (National Instruments) where any portion of 128-elements can be chosen, steered and focused to produce the desired beam in the needed locations.

RESULTS OF TASK 3:

PRELIMINARY ANIMAL EXPERIMENTS

A total of 5 Fisher rats were injected with the cancer cell line of 9L/Laz (gliosarcoma). The rats had been monitored for about 2 weeks before the tumors grew to about 1 cm in size. A bendable ring-probe was made to house the NIR sources and detectors around the tumor regions. The tumor region enclosed by the NIR probe was covered with ultrasound coupling gel, and immersed in the gel was the ultrasound transducer tip placed above the tumor region. A picture of the setup is shown in Fig. 1(a). After the NIR signals were stabilized (free of rat motion), the ultrasound excitation was initiated and the transmission of the acoustical wave was administered on the tumor region while the NIR system picked up any changes in oxygenation with/without insonfication.

The rats were administered with different ultrasonic intensity levels by adjusting the voltage and the pulse repetition frequency to see how these two system parameters would affect the oxy/deoygenation measurements. Clear changes were observed consistently in two rats, however, it was hard to draw conclusions since the other three rats did not produce any observable changes. What could be verified from the data was that when the ultrasound pulses of 180V p-p and 2KHz pulse-repetition-frequency (PRF) were applied, NIR signal changes occurred during earlier cancer stages of 1 cm in size but not in the later cancer stages. One such change is shown in Fig. 1(b).



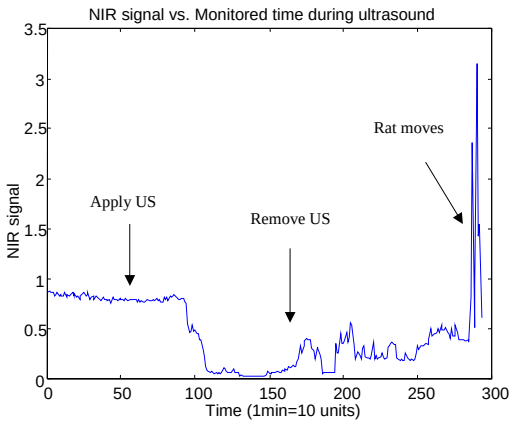


Figure 1(a). The animal experimental setup.

(b) NIR signal before and after ultrasound was applied.

Preliminary studies with 5 tumor-bearing rats demonstrate that ultrasonic vibrations can either generate significant effects (early stage tumors) on optical measurements or no effects on optical measurements (late stage tumors).

The ultrasound system used to conduct the preliminary animal experiments was transmission only. The acoustic pulses of various energy levels were delivered to the tumors and the acoustic induced effects were measured optically. However, the optical measurements were not directly proportional to the acoustic energy and were significantly modulated by the tumor **tissue elastic** condition. This is probably why we observed significant changes for two early-stage tumor rats and no changes for the late stage tumor rats.

To directly measure ultrasonic pressure effects, we have constructed a 64-channel transmission/receiving ultrasound system. The system was designed for real-time RF data acquisition, which could be used to calculate the mechanical strain generated at a tumor spot. The transducer used has 64-elements with a center frequency of 3.5 MHz. This transducer is more optimal compared with the 7.5 MHz transducer used in the initial experiments [10]. The picture of our in-house ultrasound transmission/receiving system is shown in Fig.2.

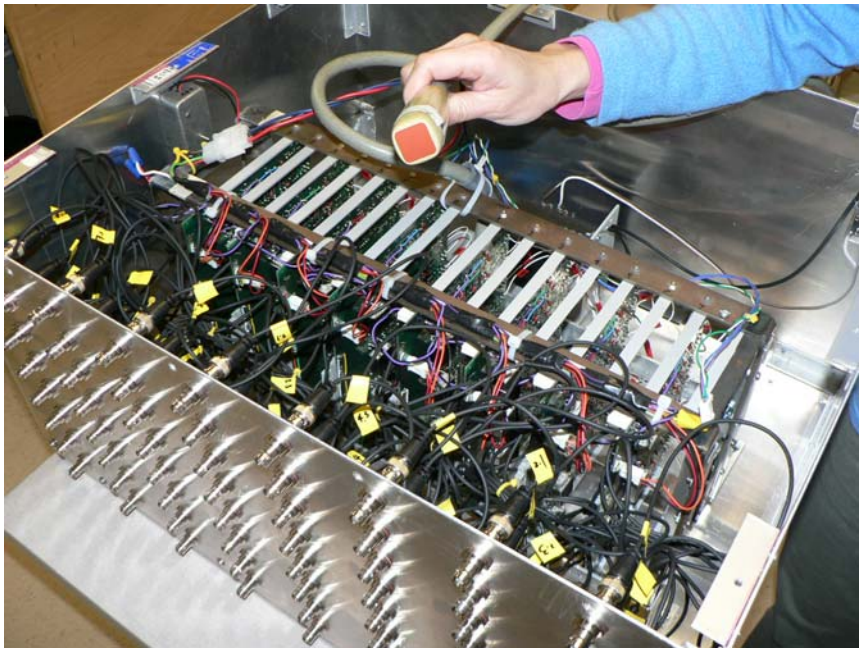


Fig.2. Our ultrasound transmission/receiving system, which is capable of transmitting with variable voltage and pulse repetition frequency and parallel reception.

Another potential source of the varying experimental results could be attributed to the difficulty in obtaining good contact between the rat tumor (grew on bony flank), ultrasound transducer, and optical laser sources and detectors. We have designed the following experimental set-up by placing the tumor-bearing rat in the middle of the tank filled with acoustic coupling gel. US transducer can be mounted on top of the tank to radiate the sound wave to the tumor. Optical source and detectors can be mounted on a circular ring, approximately 90 degrees with respect to the ultrasound transducer, to ensure optimal detection. The modified experimental set-up is shown in the figure below and it is expected to overcome the poor contact problem previously encountered

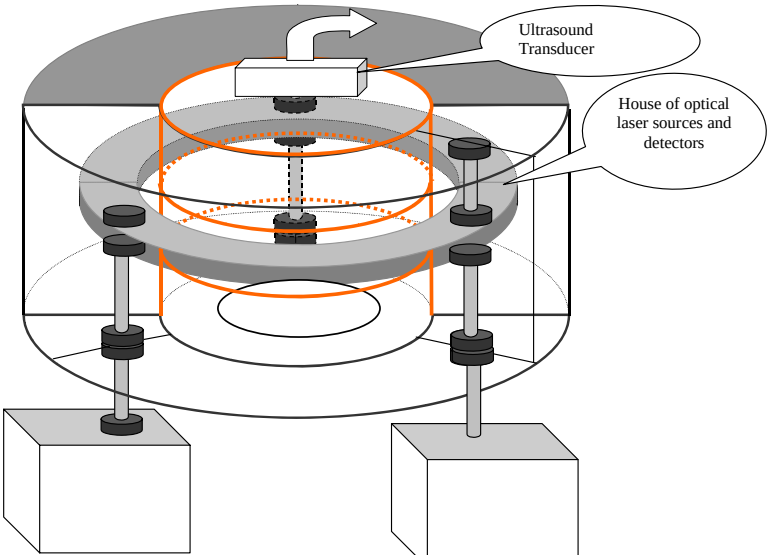


Fig.3. New set-up for improving contact between the rat tumor, ultrasound transducer and optical sensor.

Since tumor cell-line preparation and growing tumor rat require help from the University of Connecticut animal facility, we have to piggyback this experiment with a photo-acoustic animal experiment, which has funding for the needed resources. The photo-acoustic system is currently under intensive testing and we expect that we will move to experimental phase very soon.

Key Research Accomplishments

- Built a new diffusive NIR imager with multiple channels and three wavelengths;
- Calibrated and optimized the optical imagers for oxyHb and deoxyHb measurements;
- Optimized the instrumentation of the ultrasound system;
- Studied ultrasound system parameters toward maximum oxygenation changes;
- Obtained initial animal experiments and observed the ultrasound-induced optical signal changes

Reportable outcomes:

[1] N. G. Chen, D. Piao**,** H. Xia, and Q. Zhu, "Portable multi-channel multi-wavelength near infrared diffusive light imager," SPIE Photonics West 2003, San Jose, January, 2003.

[2] M Huang, T. Xie, N. G. Chen, D. Piao, and Q. Zhu, " 2-D NIR image reconstruction with ultrasound guidance," Proceedings, 2002 IEEE International Symposium on Biomedical Imaging, pp1031-1034 (Washington D.C., 2002).

[3] Chen, NG, Huang, M, Xia, HJ, Piao, DQ, Cronin, E, and **Zhu, Q**, "Portable near infrared diffusive light imager for breast cancer detection,**"** Journal of Biomedical Optics, May/June issue, 504-510, 2004.

Training accomplishments

The original PI, Dr. Daqing Piao, has completed his Ph.D degree in 2004. After completing one-year post-doctorial fellow at Dartmouth College, he joined the Bioengineering Program of the Oklahoma State University as an Assistant Professor in 2005, and is continuing breast cancer research as well as initiating prostate cancer research.

The current PI, Anastasios Maurudis, is working toward Ph.d degree with good progress.

Conclusions: In summary, we have accomplished the proposed tasks and obtained initial results.

Initial results indicate that ultrasonic vibrations at the tumor sites could either generate significant effects (early-stage tumors) on optical measurements or no effects on optical measurements (late-stage tumors). This is likely be explained by the tumor elastic properties. At early stage, the tumor tissue has similar elastic properties compared with the normal

tissue. The acoustic wave or the mechanical wave can modulate the vasculature of the tumor and produce observable changes on optical signals which are related to the hemoglobin concentration in the blood. At the later stage, the core of the tumor forms necrosis or dead tissue. The acoustic wave is unlikely to modulate the dead tissue with significant effect. Another issue is the ultrasound modulation frequency. We used existing ultrasound transducer of a higher central frequency 7.5Mhz. More evidence in the literature indicates that low frequency at 1Mhz range may produce more pronounced tissue modulation effect. We will further explore the optimal ultrasound modulation frequency in our next studies. We appreciate the DOD support to this work which provided us with evidence of ultrasound modulation of tumor vasculature.

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