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In vivo Optical Spectroscopy of Acoustically Induced Blood Stasis

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Abstract: Ultrasound-induced blood stasis has been observed for more than thirty years. Most of the literature has been focused on the health risks associated with this phenomenon and methods employed to prevent stasis from occurring during ultrasound imaging. To date, experimental observations have been either *in vitro* or invasive. The current work demonstrates ultrasound- induced blood stasis in murine tumor and nontumor tissue, observed through noninvasive measurements of optical spectroscopy, and discusses possible diagnostic uses for this previously undesirable effect of ultrasound.*

I. Introduction

Stationary sound waves have long been known to create banding effects when solids are suspended in liquids; sand in air (in a cylinder), bubbles in water, etc. In 1971, Dyson et. al. reported that stationary ultrasound waves can create bands of red blood cells ***in vitro***, using chick embryos removed from the egg shell but kept alive in saline solution [1]. Later ter Haar and Wyard showed that the banding was due to the standing pressure wave created by the ultrasound [2]. Nyborg later demonstrated that even a traveling pressure wave, with small amounts of reflection at the tissue boundaries can cause banding of blood cells in the plasma medium [3]. Many have continued to study the diagnostic limits and dangers of ultrasound and ultrasound- induced stasis [4-7], but to the best of our knowledge, no one has investigated the diagnostic potential.

A limiting factor in studying this ultrasound-induced phenomenon has been the difficulty of measuring the blood flow alterations. Previous work have required the blood vessels to be dissected from the abdomen of mice [5] or the removal of chick embryos from their shells [1] so as to be seen with microscopes and stereoscopes. The phenomenon has only been observed invasively and only in a few vessels immediately on the tissue surface or in vessels separated from the surrounding tissue. Methods have been suggested to avoid prolonged blood stasis during diagnostic imaging [4] and ultrasound intensity limits have been established to avoid tissue damage and to allow the blood flow to rebound. The current experiments have been conducted within the FDA therapeutic ultrasound limits (SPTA = 0.720 mW/cm^2) and blood stasis and banding have been observed to be reversible under these conditions.

It has been shown that oxy and deoxyhemoglobin have signature absorption and scattering effects visible in steady-state broadband diffuse reflectance optical spectroscopy [8]. Furthermore, oxyhemoglobin saturation can be determined using spectroscopic measurements of light reflected from tissue and analyzed with the diffusion approximation or the higher order P_3 approximation [9-10]. Spectral analysis performed with a P_3 approximation fit has been shown to be sensitive to the dynamic changes of hemoglobin oxygen saturation due to changes in oxygen content of air being inhaled by mice [11]. The current experiments combine the above techniques, focused standing wave ultrasound-induced blood stasis and optical spectroscopy to develop a noninvasive imaging tool with potential use in tissue diagnostics.

Cells require a constant supply of oxygen for metabolic processes. Normally, as the cells consume oxygen, hemoglobin molecules in the blood continually replenish the oxygen supply as the blood flows through the vessels. When standing wave ultrasound is used to slow or stop the blood flow, the oxyhemoglobin saturation decreases as the available oxygen is depleted. When the blood flow is stopped or slowed for short periods of time, the oxyhemoglobin saturation can be observed to decrease, using optical spectroscopy measurements, and return to pre-ultrasound levels shortly after the ultrasound radiation is stopped.

The processes involved in this phenomenon are neither simple nor straightforward and many physiological questions remain unanswered concerning the ultrasound-induced effects. Although the ultrasound intensities employed have been shown to create very little heating of the tissue and have not been shown to damage tissue, the effects of ultrasound on vessel diameter have yet to be addressed, i.e. does standing wave ultrasound constrict or expand the vessels? High intensity traveling ultrasound waves have been shown by Dalecki to exert pressure on the walls of frog heart cavities [12]. The pressures required to cause banding in moving blood are much lower than the intensities needed to deform the tissue of the heart. The current experiments were designed to remain below tissue heating, tissue damage and tissue pressure thresholds and below the current FDA limits of diagnostic ultrasound intensities.

II. Methods And Materials

Ultrasound was generated by a ≈1 MHz piezoelectric ceramic crystal (Channel Industries) mounted behind a concave aluminum lens with a focal length of 7 cm. At 1

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MHz the -6 dB focal zone diameter was 2 mm and the focal zone length was 30 mm. The ultrasound signal was created by a function generator (Agilent 33250A) and amplified by an RF amplifier (Amplifier Research 25A250A), monitored and recorded by an oscilloscope (Tektronix TDS 2022). The ultrasonic field was measured and characterized using a hydrophone (Onda Co. HNR 500). The intensity of the ultrasound was maintained at Spatial Peak Temporal Average Intensity (SPTA) $\approx$ 0.7 mW/cm^2, averaged over the burst cycle.

Initial tests of the ultrasound setup included a repeat of Dyson's seminal experiment, but with lower acoustic intensities (SPTA = 0.7 mW/cm^2) and lower frequencies ($f \approx$ 1 MHz). The ultrasound was observed visually to stop blood flow, causing bands to form for short periods of time. In order to perform a non-invasive test regarding the efficacy of the ultrasound in the mouse leg, a laser Doppler system

source was obtained using a diffuse reflectance standard. The optical signal then was cropped to avoid the spectral regions of low light (< 400 nm) and regions near the end of the spectrometer's sensitivity (> 1000 nm). Ultimately, the spectra were cropped to regions between 475 nm and 650 nm where significant changes in optical absorption are present due to oxy/deoxyhemoglobin shifts and few other absorbers affect this region of the spectrum. Several isolated wavelengths were initially considered (515 nm, 528 nm, 540 nm, 560 nm, 579 nm and 578 nm), but eventually the ratio of intensities (I) at two wavelengths, 560 nm and 540 nm, was chosen. The ratio of I_{560}/I_{540} has been shown to be significantly affected by the presence (or absence) of the ultrasound. Since the intensities at 560 nm and 540 nm are dependent upon the oxy/deoxyhemoglobin saturations, it can be demonstrated that the ratio I_{560}/I_{540} correlates to