High frequency ultrasound scattering from mixtures of two different cells lines: tissue characterization insights

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Abstract*— Ultrasound imaging in the most commonly used imaging modality in medicine today. Ultrasound images are based on the echoes received from scattering structures in the human tissues. However, these images are traditionally based on the intensity of the received ultrasound echoes, discarding the information that is present in the frequency dependence of the backscattered waves. In this paper we demonstrate how high frequency ultrasound (20MHz) is particularly sensitive to the sizes of the nucleus (or cells) in tightly packed cell aggregate models (cell pellets) and how the frequency dependence of the backscatter provides information about the size of the effective scattering structures in an inhomogeneous medium composed of two different cell lines. Two cell lines with distinct sizes (AML 11 µm, PC3 23 µm) were used and mixed together in different portions according to volumetric ratios. A VisualSonics VS40-B high frequency ultrasound imaging device, with full access to the radiofrequency (RF) data, was used to collect images of the cell pellets and the rf data associated with those images. Spectral analysis techniques were used to measure the spectral slope, mid-band fit (MBF) and intercept of the normalized power spectrum of the rf data. It was found that the PC3 cell pellet had a much greater scattering strength as determined by the MBF compared to the AML cells (-38dB vs. -54dB), even though the smaller AML cells have a cell number density (number of cells per unit volume) 5 times greater. Moreover, the spectral slope of the PC3 cell pellet was considerably smaller than then the spectral slope of the AML pellet (0.55 dBr/MHz vs. 0.91 dBr/MHz). Both these results are consistent with scattering theory when taking into account the sizes of the nuclei and cells. Spectral parameter values of the mixtures of the AML and PC3 cells were in-between the values obtained with the pure cell pellets. The work demonstrates the sensitivity of high frequency ultrasound spectroscopy to the cell nucleus size.

Keywords-tissue characterization; ultrasound scattering; cell pellet model; cell scattering; spectrum analysis

I. INTRODUCTION

Ultrasound imaging is the most common imaging modality providing images of human anatomy that are based on the soft tissue echogenicity variations. Typically, only the backscatter signal intensity is used to create an image. Quantitative ultrasound methods have been developed to provide information about tissue microstructure unavailable in typical intensity-based ultrasound images. Methods that depend on the analysis of the ultrasonic radiofrequency (rf) spectrum, pioneered by the late Frederic Lizzi at the Riverside Research Institute, rely on the frequency dependence of the rf backscatter to infer properties of tissue microstructure [1-3]. Our group has utilized these methods to analyze the backscatter from cells using high frequency ultrasound imaging [4, 5]. We hypothesize that as the ultrasound wavelength approaches the size of the cell, it is more sensitive to cell structure and changes in the cell structure due to treatment effects. Our work has shown that in a cell pellet model (a compact aggregate of cells that emulates the cellular organization of tumors), the nucleus size, not the cell size, best correlates with the ultrasound backscatter frequency dependent parameters [6]. The sensitivity of ultrasound scattering to nucleus size is of great interest since one of the most common features of eukaryotic cells is the tight control of the cytoplasm to nucleus diameter [7, 8]. Pathologies therefore that result in a change in nuclear diameter could potentially be detected using high frequency ultrasound. For example, one of the most common ways to diagnose a cancer is to analyze the variations of the nuclear size and morphology in cells [9]. To explore the sensitivity of ultrasound to different populations of cells with different diameters, in this work we explore the changes in ultrasound backscatter in the cell pellet model using two different populations of cells that are mixed in different proportions.

II. METHODS

A. Biological Materials

Two cells types, Acute Myeloid Leukemia (AML) cells (~10 micron diameter) and PC3 cells (human Prostate Cancer cell line, ~23 micron diameter) were imaged with a high frequency ultrasound imager (VS40B, VisualSonics Inc. Toronto, Canada) that could store the rf signals associated with an image. Compact aggregates of cells were created by centrifugation (creating a cell pellet). Cells obtained from frozen stock samples were cultured at 37°C in 150mL of alpha minimal essential media (Invitrogen Canada Inc.) plus antibiotics (100 mg/L streptomycin (Bioshop, Canada), 100 mg/L penicillin (Novapharm Biotech Inc., Canada)) with 5% fetal bovine serum (Cansera International Inc., Canada). Cells

were harvested by centrifugation at a centripetal force of 960 g for 6 minutes at 4°C. Minimum essential media (MEM) was aspirated and cells washed in phosphate buffered saline and centrifuged again at 2000 g for 10 minutes to form the final pellet to be imaged. Pellets were made either of pure populations of the two cell lines, or by mixing the cell populations in different proportions (ranging from 1/128 to 1/2 of the cell volume comprised of prostate cells, in steps of 1/2, with the remaining volume containing AML cells). Hematoxylin and Eosin (H&E) staining confirmed the appropriate mixing of PC3 cells in the pellets created, with no obvious clustering in the cell pellet (Figure 1).

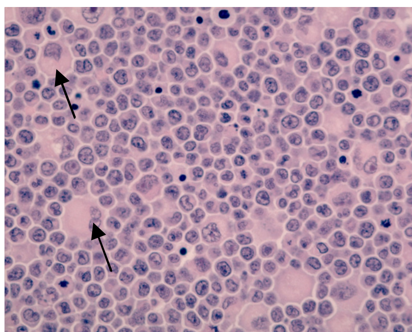

Figure 1: H&E Histology of 1/8 pellet (1/8 of the total volume occupied by PC3 cells, the rest AML cells). Larger cells are the PC3 cells (shown by the arrows).

B. Ultrasound imaging

A f-2.35 transducer with central frequency of 20 MHz and 10-30MHz bandwidth was used. The transducer was attached to the VS40 ultrasound imager. The VS40B has the ability to select regions of interest from the B-scan images and store the raw radio-frequency (rf) data associated with the region of interest (ROI). The sampling frequency of the A/D board is 500MHz. To analyze the data from the pellets, a ROI was centered at the transducer focus and was approximately 1 mm in length and several mm in width, collected several hundred microns from the pellet surface. The Fourier transform of the ROI was taken using a Hamming apodization function. The power spectrum was obtained by averaging the results from at least 200 independent scan lines from 3 different planes in the pellet (separated by at least one beam-width, approximately 200 µm). The power spectrum was divided by the power spectrum of the echo from a calibration target to obtain the normalized spectra (a flat polished fused silica crystal (Edmund Industrial Optics Inc., Barrington, NJ, USA, part 43424). The perpendicular reflection off the quartz flat located at the focal point of the transducer was used to remove system and transducer transfer functions. Linear regression analysis was applied to the calibrated spectral amplitudes to calculate the spectral slope, mid-band fit and intercept [5].

III. RESULTS

Hematoxylin and Eosin (H&E) staining confirmed the appropriate mixing of PC3 and AML cells in the pellets, with no obvious clustering observed for the pellets for which H&E was obtained (Figure 1). Further evidence of the mixing uniformity is the uniform speckle intensity over the entire pellet during the ultrasound imaging experiments (Figure 2). As can be appreciated in Figure 1, the PC3 cells are significantly larger. Separate measurements showed that the AML cells (11.5 +/-1.3 µm cell diameter, 9.1 +/- 1.2 µm nucleus diameter) are smaller than the PC3 cells (22.6 +/- 5.4 µm cell diameter, 15.6 +/- 4.5 µm nucleus diameter).

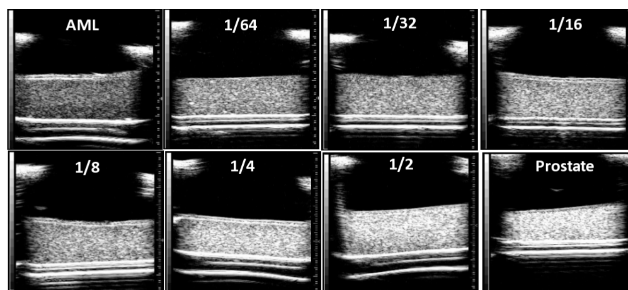

Figure 2: Ultrasonic images of cell pellets of a popultaions of AML cells (top left) and PC3 cell (bottom right). The size of the field of view is 8 x 8mm. The number at the top incidates the volumetric portion of the cell pellet occupied by PC3 cells, as desribed in the methods.

The ultrasound images of the cell pellets are shown in Figure 2. The numbers in the top of the images represent the volume occupied by PC3 cells. Even at these high frequencies, individual cells cannot be resolved and therefore a speckle pattern is formed for all of the pellets. The ultrasound backscatter from cell pellets containing the larger PC3 cells was significantly larger compared to the smaller AML cells (mid-band fits of -38dBr vs. -54dBr, respectively). This is despite the fact that simple volumetric calculations show that there should be more than 5 times more AML cells per unit volume when compared to the PC3. If each cell had the same scattering cross section, one would expect the AML cell pellet to scatter more ultrasound due to the greater number density (assuming no packing effects [10]). This suggests that scattering strength is highly sensitive to the nucleus (or cell) size. Pellets with mixtures of cells had MBF values in-between these two values, as one might expect (Table 1). Moreover, the speckle intensity increased with the increasing proportion of PC3 cells. This result can be expected due to the large size of the PC3 cell nucleus.

The spectral slopes were significantly reduced for the PC3 cells (0.55 dBr/MHz) compared to the AML (0.91 dBr/MHz). The experimental normalized power spectra curves are presented in Figure 3, and the best fit linear regression lines are shown as the thin lines in the figure. Again, scattering theory predicts that the spectral slopes are smaller for cells that have scatterers that are larger [2, 11]. The values for the mid-band fits and spectral slopes of the mixed cell pellets ranged between these two extremes. The mid-band fit increased by 1-3 dBr and the spectral slope decreased by 0.05 dBr/MHz for every doubling in the volume composition of PC3 cells in the cell pellet (starting at 1/128 of the cell pellet volume occupied by

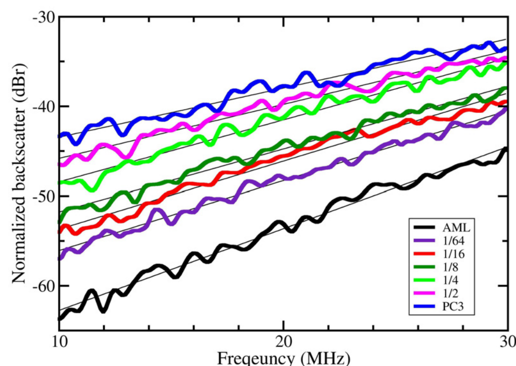

Figure 3: Ultrasonic images of cell pellets of a popultaions of AML cells (top left) and PC3 cell (bottom right). The size of the field of view is 8 x 8mm. The number at the top incidates the volumetric portion of the cell pellet occupied by PC3 cells, as desribed in the methods.

PC3 cells, Table 1). It is therefore clear that the nucleus or cell size significantly influence the ultrasound backscatter, as both the backscatter strength and its' frequency dependence show large variations which are consistent with theory. A more rigorous analysis of these results (in which factors such as the attenuation are taken into account and an analysis based on the scattering theory of Lizzi et. al.) is the subject of a future manuscript. It is clear however that in this experimental system composed of biological cells, the frequency dependent ultrasound backscatter critically depends on the size of the cell / cell nucleus.

IV. CONCLUSIONS

The data demonstrate the effect of cell size on ultrasound backscatter in this model system. Despite the fact that there are many more AML cells per unit volume, the scattering strength of the larger PC3 cells dominate the backscatter signal, increasing the scattering strength and reducing the spectral slope. The changes in the spectral slope and backscattering strength are consistent with predictions of theoretical models of ultrasound backscatter based on analysis of the normalized rf-spectrum. This work may lead to better ultrasound imaging strategies for clinical examinations, especially when the size of the nucleus is an important factor. One example for which the authors have considerable experience is in cancer diagnosis and treatment monitoring for which the size of the nucleus is an important factor.

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TABLE I. SPECTRAL PARAMETERS FROM CELL PELLETS

Cell pellet mixture	**Spectral Parameters**		
	Spectral slope (dBr/MHz)	***Mid-Band fit (dB)***	***Intercept (dB)***
100% AML	0.91	-53.6	-71.9
1/128 PC3	0.86	-47.4	-64.6
1/64	0.78	-48.3	-63.9
1/32	0.80	-47.0	-63.3
1/16	0.76	-46.1	-61.3
1/8	0.70	-44.8	-58.8
1/4	0.69	-41.5	-55.4
1/2	0.60	-39.8	-51.9
100% PC3	0.55	-38.0	-49.0

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