New Insights into High Frequency Ultrasonic Tissue Scattering

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1. Introduction

We have shown that high frequency ultrasound (HFU) backscatter is sensitive to the structural and physical changes that cells undergo during cell death[1-2]. For example, during apoptosis induced by exposure to the chemothrapeutic cisplatin the ultrasonic backscatter amplitude of Acute Myeloid Leukemia (AML) cell pellets increases by a factor of 4 creating a large contrast between treated and untreated tissue, accompanied by subtle changes in the slope of the normalized backscatter spectrum (spectral slope) of the ultrasound radiofrequency (RF) signals [2]. This ultrasonic contrast between normal and damaged tissues has also been seen in-vivo and in other models of tissue damage/remodeling such as mammary tissue involution and organ preservation [3-4]. We are investigating clinical applications of ultrasonic detection of cell death, which would provide a rapid non-invasive and inexpensive way to assess cancer treatments. Our working hypothesis is that for high frequency ultrasound the backscatter characteristics from aggregates of cells in tumor tissue are closely linked to cell structure and in particular the nuclear size and its acoustic properties. Furthermore, we hypothesize that the changes in backscatter observed with treatment or tissue remodeling are due to the structural changes that occur during cell death. In this work we present data that demonstrate how ultrasound backscatter changes with ultrasound interrogation frequency for the same biological scattering model. Moreover, we will present some of our recent work using acoustic microscopy to elucidate the acoustical properties of cells at the single cell level.

2. Materials and Methods

Data were collected from compact aggregates of cells (from now referred to as cell pellets) of AML cells. The spatial distribution and packing of these cells emulates the spatial distribution and packing cells in tumors. 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. Histological examination revealed that the packing of cells in the pellet emulated that of tumor xenografts of the same cells when injected in the hind leg of mice.

The cell pellets were imaged and RF data were collected with two different ultrasound imaging devices for which RF data could be accessed and analyzed: a VS-40B imager (VisualSonics Inc., Toronto, Canada, www.visualsonics.com) and a Ultrasonix RP (Ultrasonix Medical Devices, British Columbia, Canada, www.ultrasonix.com). The VS-40B imager was used with two broadband transducers were used to image cell pellets: one with a center frequency of 20MHz (8.5mm diameter, f2.3, –6dB bandwidth: 12-30MHz) and the other 40MHz (3mm diameter, f2, –6dB bandwidth: 20-53MHz). The Ultrasonix RP device was used with a L 14-5/38 linear array probe operating at a central frequency of 6 MHz.

Acoustic microscopy data were collected from either polystyrene microspheres (Beckman Coulter Inc.) suspended in gelatin phantoms or single cells that were immersed in PBS. The acoustic microscope used was the SASAM 1000 (Fraunhofer Institut für Biomedizinische Technik, St. Ingbert, Germany), which is a time-resolved acoustic microscopy system in which the echo signal from a substrate is recorded by using a short pulse instead of a long burst pulse usually used in acoustic microscopy systems. The ultrasound transducer is mounted on an optical microscope, allowing the near simultaneous optical and acoustical imaging. To achieve this, an inverted optical microscope Olympus IX81 (Olympus, Tokyo, Japan) has to it attached a scanning unit attached to a rotating column that allows switching between the condenser and the acoustical lens.

3. Results and Discussion

3.1 Cell Pellet Imaging

The RF lines and the images of an AML cell pellet at 6MHz, 20MHz and 40 MHz are shown in Fig.1. The top row of the figure contains data collected with the Ultrasonix (6MHz), the middle row data from the VS-40B using the 20MHz transducer whereas the data collected using the VS-40B at 40MHz are shown at the bottom of

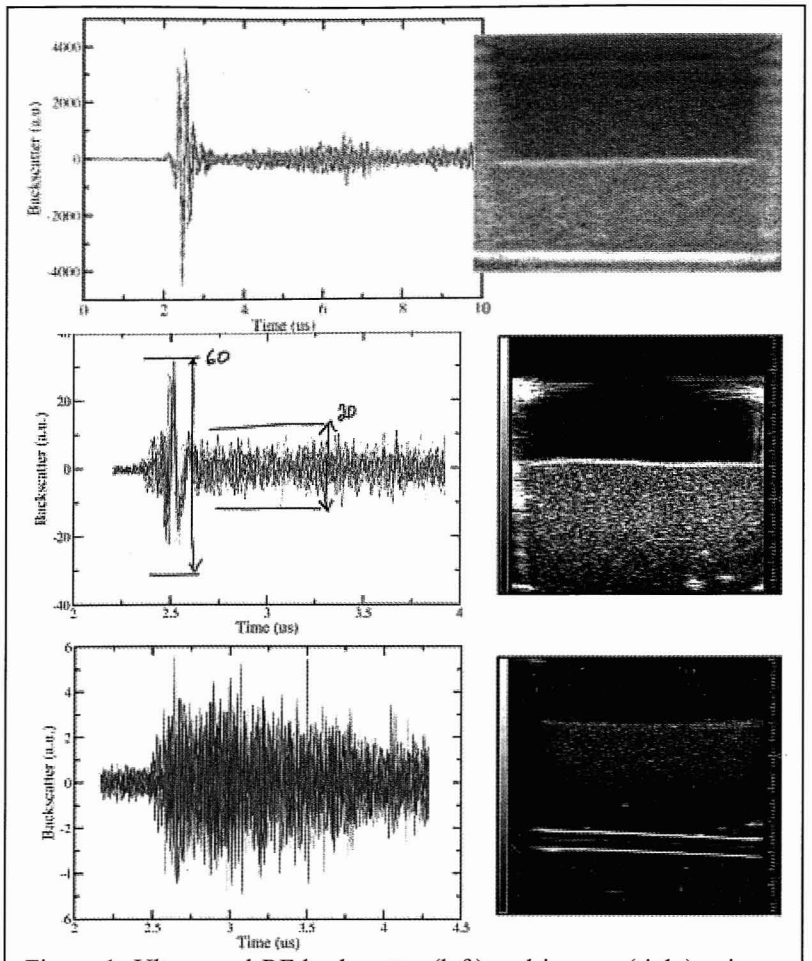

Figure 1: Ultrasound RF backscatter (left) and images (right) using a 6MHz transducer (top) a 20MHz transducer (middle) and a 40MHz

the figure. The left column of figure 1 has multiple traces of RF lines, superimposed, to provide a visual representation of the RF data collected at different locations within the image and at the various frequencies. The y-axis is in arbitrary units, whereas the x-axis is in microseconds. The right column of Figure 1 shows the ultrasound images of the cell pellets. The container that encloses the cell pellet can be distinguished by the reflections at the edges of the container for the first two images. The pellets, depending on the frequency, have a strong reflection at the interface between the cells and the coupling medium. Moreover, a speckle pattern can be seen in these images as even at the highest frequency in these experiments individual cells cannot be resolved. One of the main features of these RF lines is the relative strength of the reflection from the surface of the AML cell pellet compared to the signal from further depths within the pellet. It can be appreciated that at the lower frequencies the signal from the pellet surface is much stronger that the signal from further depths within the pellet, whereas at 40MHz the surface reflection is barely discernable compared to the signal from further depths. At the lower frequencies, due to the large ultrasound wavelengths compared to the size of the cells (the cells are 10 μm in diameter, and the ultrasound wavelength is approximately 250 μm at 6MHz) the pellet is more like a homogeneous medium and a large impedance mismatch that occurs at the surface of the pellet causing the specular-like reflection. However, at 40MHz, the wavelength is 37 μm which is approaching the physical size of the cell. Therefore, the surface reflection is more like diffuse scattering, and the scattering is similar in strength to that seen for further depths within the pellet. This also suggest as to why high frequency ultrasound is required to provide adequate sensitivity to distinguish features on the order of the cell size: in this example, it is clear that at 6MHz only boundaries that enclose these cells can be distinguished. This is likely one of the reasons that ultrasound tissue characterization does not have much discriminatory power at the clinically relevant frequencies and has not met with the commercial success that was anticipated by the community when such methods were initially released: it may have not been sensitive to the scattering structures of interest.

3.2 Acoustic Microscopy

Our laboratory recently started investigations of ultrasound scattering at the single cell level, to determine the changes in individual cell ultrasonic properties as a function of treatment. This requires much higher ultrasonic frequencies to provide the adequate resolution. Such a system was commissioned in December of 2007: the SASAM 1000. The first attempts were to image polystyrene microspheres, and to determine whether the resonance patterns our lab has measured before in the 20-60MHz range[5-6] can be measured with this system (using the appropriate ka). Figure 2 shows the acoustic researcher interface to the SASAM 1000. In this particular example, polystyrene microspheres of 10 μm size were imaged at 400MHz. The left side of the interface shows a c-scan image of the beads (bright circles) that can clearly be distinguished from the surrounding gel. Moreover, the RF data for one plane (as defined by the yellow horizontal line) are shown in the image below the c-scan. The horizontal line pattern that expands downwards is related to the scattered wave from the bead. The resonant scattering pattern can be appreciated in this figure. If one were to chose only one

RF line (defined in location by the yellow crosshair of the middle left image), the RF line acquired is shown to the right. Even though the pulse sent was short, the scattered wave is longer in time due to the resonant scattering of the bead. Currently we are performing theoretical simulations to determine whether these patterns are the ones anticipated for this size range and physical bead property.

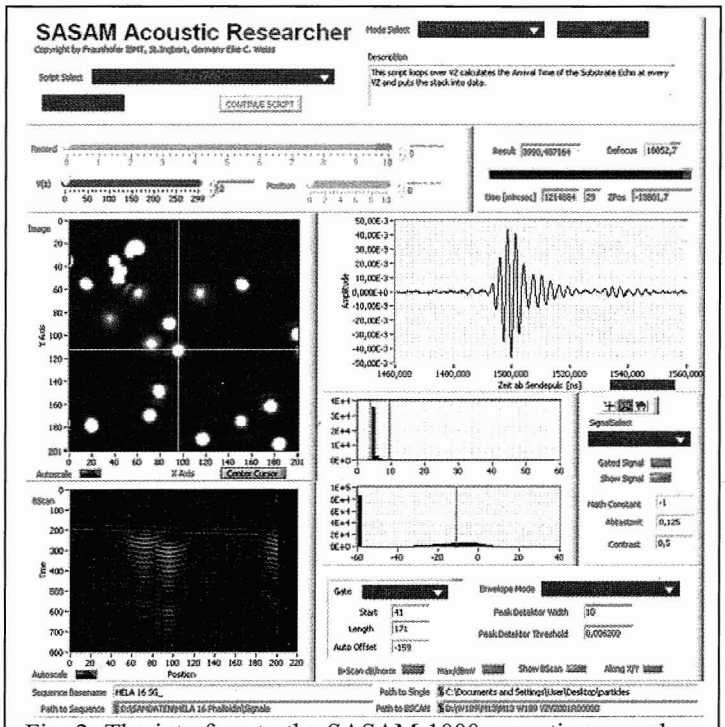

Fig. 2: The interface to the SASAM 1000 acoustic researcher. In this example 10 μm size beads are imaged in c-scan mode (middle left). Several beads are in the field of view. The RF data are displayed below the image for one plane (the horizontal yellow crosshair in the image above). A single RF trace, acquired at the location of the crosshairs is shown in the figure to the right. The resonance pattern of the bead can be distinguished.

4. Conclusion

We have imaged cell pellets using frequencies ranging from 6MHz to 40MHz. The cell pellets are a model system for scattering from solid tumors with high cellular content. It can be seen that the scattering from cell pellets is highly dependent of the interrogating frequency, which dictates the level of interaction of the ultrasound with the cells. At low frequencies (6MHz) the cell pellet was more like a homogeneous medium and therefore the surface reflection dominated the signal. At higher frequencies (40MHz), the cell pellet surface reflection was on the order of the reflections (scattering) related to the wave propagation deeper within the pellet. This example illustrates why clinically relevant frequencies have not been very successful in tissue characterization: the scattered wave are not as sensitive to features at the scale of the single cell level. Finally, using very high frequency ultrasound (400MHz), we have shown the resonant scattering patterns that a 10 μm size bead produces. The patterns are similar to what we have seen in the 20-60MHz range.

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