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The Effect of Volume Fraction on the Backscatter from Nucleated Cells at High Frequencies

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Abstract — Small variations in scatterer volume fraction, which can result from changes in tissue microstructure due to cancer therapies or organ preservation, may have a significant impact on ultrasound backscatter. Although the effect of volume fraction has been studied for non-biological scatterers and red blood cells, this study addresses the case of nucleated cells. Suspensions with volume fractions up to 70% of acute myeloid leukemia cells were insonified with broadband 20 MHz and 40 MHz pulses. The resultant average normalized backscatter intensities plotted as a function of volume fraction demonstrated a better agreement with the Yagi-Nakayama continuum scattering theory rather than the Mo-Cobbold particle scattering model (using hard sphere packing). Normalized backscatter increased with cell volume fraction up to a maximum value, occurring between 20 and 30% volume fraction, varying with frequency and then decreased with further increases in volume fraction. This result may have implications in the development of new quantitative, ultrasound-based tissue characterization techniques.

Keywords – backscatter, volume fraction, nucleated cell

I. INTRODUCTION

Recently, new high frequency (>10 MHz) ultrasound devices have emerged with better system signal-to-noise ratio characteristics. These devices make it possible to measure ultrasound scattering in tissues so that small variations in scatterer volume fraction, which can result from changes in tissue microstructure due to cancer therapies or organ preservation, may play a role in the changes in backscatter that are observed. In the case of organ preservation, as allografts

preserved for transplantation decay over time, it has been observed that ultrasound backscatter at high frequencies increases [2]. This tissue decay, due to ischemia, is manifested by various changes (see Figure 1) in cell morphology, such as variations in shape and the appearance of vacuoles in the cytoplasm, but also in overall changes in cell packing and spacing. Each of these alterations to the tissue likely affect the overall scattering response. However, the relative importance of each type of change is unknown.

The objective of this study is to investigate experimentally the effect of volume fraction, the fraction of the total interrogated volume occupied by cells, on high frequency ultrasound backscatter. Although the effect of volume fraction has been well documented for non-biological scatterers and red blood cells (RBCs) [3, 4], this study addresses the case of nucleated cells. As we have previously shown that nuclear changes have a significant effect on ultrasound backscatter [5], it is reasonable to assume that simply the presence of cell nuclei might have an impact on the relationship between volume fraction and backscatter.

II. METHODS

Suspensions, starting with a volume fraction of 70% and consecutively diluting to achieve lower concentrations, of acute myeloid leukemia (AML) cells (OCI-AML-5 cell line [6]; mean cell diameter $12.1 \pm 1.7 \mu\text{m}$, mean nucleus diameter $10.2 \pm 1.2 \mu\text{m}$, as determined by optical confocal microscopy of bisbenzamide-stained cells with ultraviolet illumination) in phosphate buffered saline solution were insonified with broadband (100% 6 dB bandwidth) 20 and 40 MHz pulses using two focused transducers (with corresponding resonant frequencies) and a VS40b ultrasound device (VisualSonics Inc., Toronto, Canada) sampling at 500 MHz. Only 11 volume fractions were selected for study to keep the experiment time at a minimum to ensure consistent cell viability between data sets.

For each suspension, the raw, unprocessed A-lines acquired from 300 independent positions were multiplied by a Hamming window (over the region of the signal corresponding to the depth of field of the transducers) and Fourier transformed. The average squared magnitude of these spectra was calculated and divided by the power spectrum from a corresponding reference; the reflection from polished SiO_2 crystal placed in the same cell suspension at the transducer focus, perpendicular to the beam. The use of different references for each volume fraction condition implicitly corrects for the variation in attenuation due

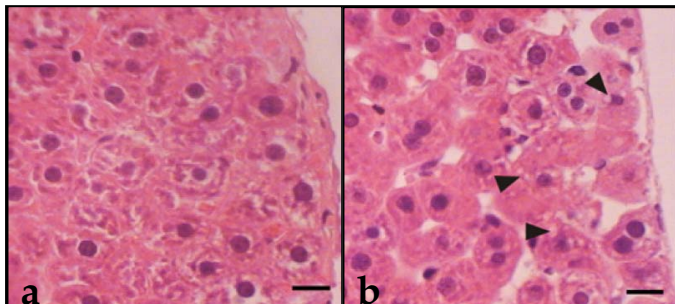


Figure 1. Hematoxylin & Eosin (H&E) staining collected after 24 hours of storage for: (a) rat livers preserved using a standard liver allograft preservation protocol, and (b) rat livers improperly preserved (not flushed with preservation solution). In addition to changes in cell shape and cytoplasm vacuolization (some sample vacuoles are indicated with triangles), there is an apparent decrease in the fraction of the total volume occupied by cells in (b). The scale bar indicates 20 μm . Figure reproduced with permission from [1].

to differing numbers of cells in the intervening region between the transducer surface and the focal plane.

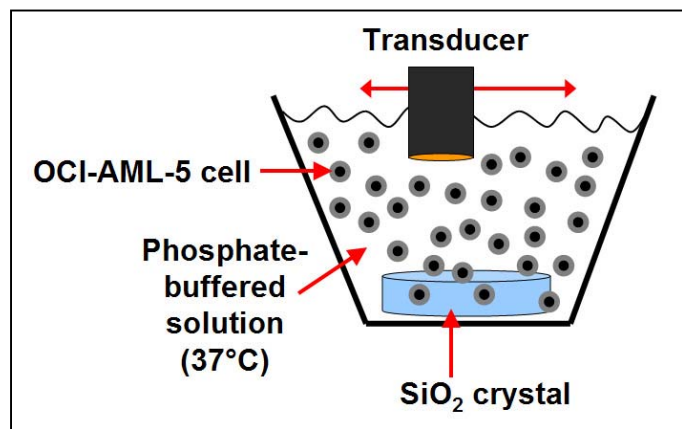


Figure 2. Experimental setup: Cell suspensions with different volume fractions were prepared by dilution of an initial 70% suspension into small vials and interrogated with a transducer submerged in the suspension.

III. RESULTS

The integrated backscatter values for each volume fraction condition, calculated over the respective 6 dB transducer bandwidths and normalized to the maximum value found across all volume fractions, are shown in Figure 3 (results using the 20 MHz transducer) and Figure 4 (results using the 40 MHz transducer). Corresponding representative B-scans and

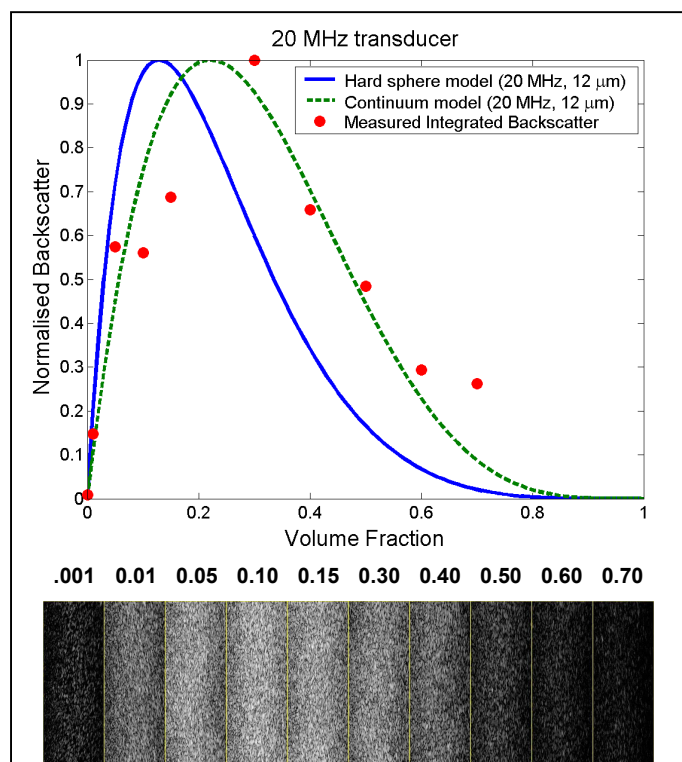


Figure 3. Effect of volume fraction on backscatter centered at 20 MHz. Above: results of signal analysis; Below: B-scans (rotated by 90°) of the transducer depth of field with consistent gain settings. Note that whereas the integrated backscatter values are corrected for attenuation, the B-scans are not.

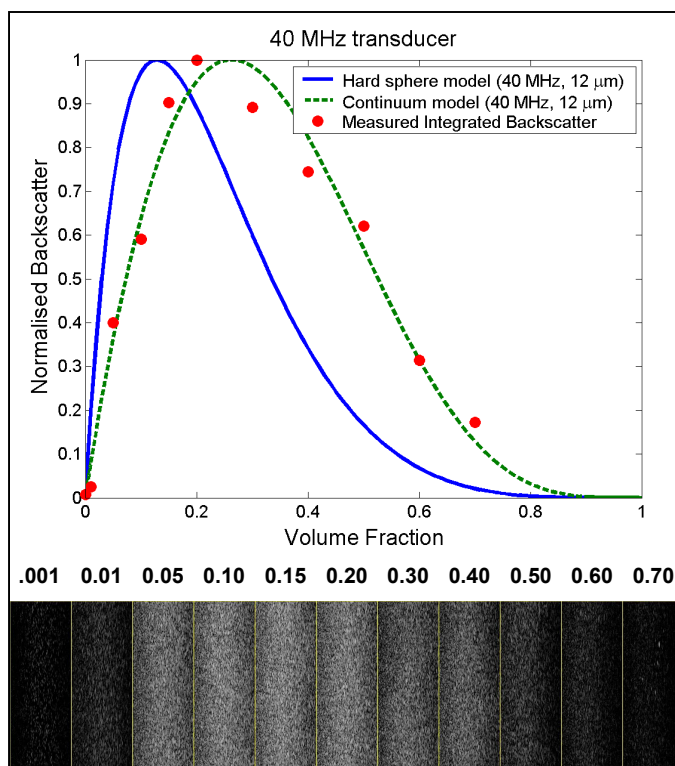


Figure 4. Effect of volume fraction on backscatter centered at 40 MHz. Above: results of signal analysis; Below: B-scans (rotated by 90°) of the transducer depth of field with consistent gain settings. Note that whereas the integrated backscatter values are corrected for attenuation, the B-scans are not.

the theoretical normalized backscatter using two classical ensemble scattering models – hard sphere and continuum – are also presented in Figures 3 and 4. The 0.20 volume fraction condition at 20 MHz was not included in the analysis, and is not shown in Figure 3, as it was subsequently discovered that a bubble was lodged in the concave transducer aperture during this acquisition set.

IV. DISCUSSION

The normalized integrated backscatter values plotted as a function of volume fraction demonstrated a better agreement with the Yagi-Nakayama continuum scattering theory [7] rather than the Mo-Cobbold particle scattering model (using hard sphere packing) [8]. Normalized backscatter increased with cell volume fraction up to a maximum value, occurring between 20% and 30% volume fraction, and then decreased with further increases in volume fraction. In comparison, studies at 7.5 MHz with stirred (to minimize aggregation) RBCs (main axis diameter 7.5 μm) indicated better agreement with the hard sphere theory, with the maximum backscatter tending to occur below 20% volume fraction (hematocrit) [3, 4]. This divergence between the results from AML cells and RBCs may be due to the presence of cell nuclei in addition to the effect from the underlying cell shape differences (AML cell: spherical, RBC: biconcave ellipsoid). No significant difference was observed between the AML cell results from the 20 MHz and 40 MHz transducer. This observation indicates that the different backscatter vs. volume fraction response of RBCs is likely not due to differing cell size or operating frequency. The

more homogeneous content of an RBC (primarily hemoglobin and water), compared to a nucleated cell, could be the reason why the backscatter from RBCs in suspension is better predicted by a simple hard sphere model.

Although both theoretical models predict that normalized backscatter should diminish towards zero as volume fractions approach 1, both data sets appear to suggest a break from this trend. In fact, it is known that these model predictions for high volume fractions must be false, since several previous studies of densely packed ensembles of AML cells (achieved by centrifugation) did yield measurable backscatter, often quite high in intensity [9, 10]. It is possible that, at these high volume fractions, the scattering effect of the nuclei begin to dominate and the increasing amount of contact between cells creates a somewhat homogeneous surrounding medium of cytoplasm. It is clear that a new model must be developed and further experimental studies performed to better understand the scattering from nucleated cells at volume fractions above 60%. In this region, there appears to be a transition from the individual cell, suspension regime to the densely packed, ensemble regime. A new ensemble scattering model using empirical measurements of scattering from single cells is currently under development [11].

This study has indicated the possibility that the presence or absence of nuclei significantly influences the effect of volume fraction on ultrasound backscatter. These experiments will be repeated with cell types that have different nucleus to cell diameter ratios and with spherical, cell-like biological structures lacking a nucleus, such as lysosomes, to better expose the role of the nucleus on the volume fraction effect.

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