THE EFFECT OF PACKING ORDER ON ULTRASOUND BACKSCATTER FROM CELLS AT DIFFERENT VOLUME FRACTIONS

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1. INTRODUCTION

Recently, new high frequency ultrasound devices have emerged with better system signal-to-noise ratio characteristics, which 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, may have a significant impact on the backscattered signal. Previous studies have shown that the backscattered ultrasound power from suspensions of scatterers is related to the fraction of the total volume occupied by the scatterers. Although this volume fraction effect has been well studied for certain biological scatterers, in particular red blood cells (Shung *et al.*, 1984; Mo *et al.*, 1994), the sensitivity of these responses to packing order, assuming no aggregation, have not been investigated. In this study, a three-dimensional computer model was used to study the effect of position randomness on the ultrasound backscatter from cell suspensions at several volume fractions.

2. COMPUTER MODEL

Although producing cell suspensions *in vitro* with any desired volume fraction is a feasible undertaking, it is impossible to create suspensions in which cells have a precise degree of freedom of position. Therefore, a computer model was developed to study the effect of these two factors on ultrasound backscatter. To calculate the backscatter frequency response from a simulated suspension of cells, the total backscattered pulse must be computed. This is calculated by summing the individual backscattered pressure pulses generated from each cell (all cells are assumed to be identical), accordingly time-delayed by depth (frequency-dependent attenuation in the medium is assumed to be negligible) and weighted by a transducer aperture function. As there is currently no satisfactory scattering model for single, nucleated cells, a representative backscattered pulse measured from a single acute myeloid leukemia (AML) cell (13.4 μm mean diameter) with a broadband 20 MHz focussed transducer (Baddour *et al.*, 2005) was used as the elemental scattered pulse (see Fig. 1).

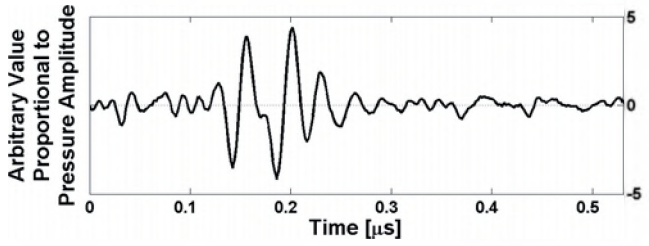

Fig. 1. Backscattered pulse from a single AML cell interrogated with a broadband 20 MHz transducer (Baddour *et al.*, 2005).

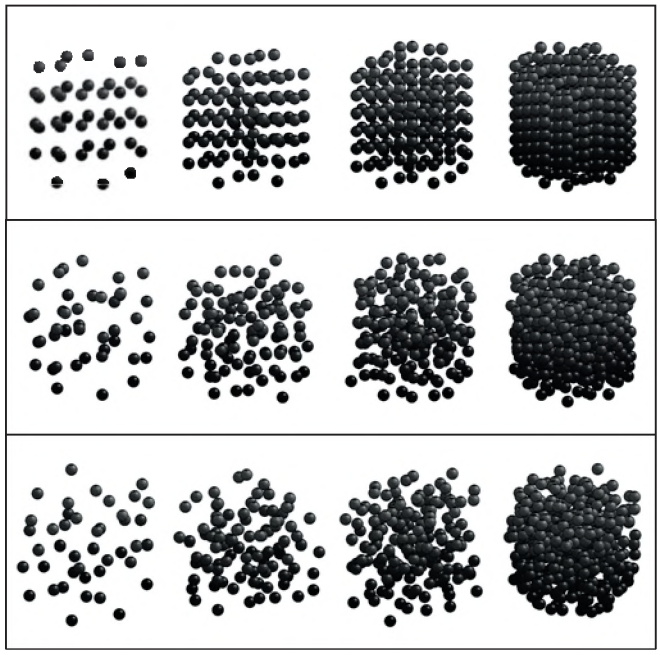

Fig. 2. Orthographic projections of realisations of some simulated cell suspensions, varying the volume fraction from left to right (0.02, 0.06, 0.10, 0.30) and varying the value of *cellLocRange* from top to bottom (0.00, 0.25, 0.50). Note that these are simply meant to be illustrative; the pack dimensions do not reflect the cylindrical region employed to obtain the simulation results.

The spatial arrangement of cells in the simulated suspensions can be varied, for example to simulate perfect, crystal-like sets or more random arrangements (some examples are presented in Fig. 2). The model extends a previously proposed two-dimensional model (Baddour *et al.*, 2002) using the parameter *cellLocRange* to control the randomness of the cell positions in the suspension. Here, *cellLocRange* is defined as a fraction of the inter-cell spacing, for a given volume fraction, assuming an ordered lattice packing. When *cellLocRange* is zero, the cells are packed in a perfectly staggered lattice. As *cellLocRange*

increases, cells are allowed to uniformly randomly take positions farther away (up to *cellLocRange* times the lattice-based inter-cell spacing) from their assigned lattice position.

3. RESULTS AND DISCUSSION

Cell suspensions were simulated for several values of volume fraction (up to 0.74, the limit for face-centered cubic packing of spheres) and *cellLocRange*. In each case, the total backscattered pulse returned by the model was used to calculate the integrated backscatter (IB) (Raju and Srinivasan, 2001). The IB was calculated for the range of 10 - 30 MHz, corresponding to the 6 dB bandwidth of the transducer employed to acquire the backscattered pulse from the single AML cell. A reflected pulse, using the same acquisition setup as the AML cell measurement (Baddour *et al.*, 2005), from a flat, polished SiO_2 crystal, placed perpendicular to the beam at the transducer's focus, was used as the reference in the IB calculation.

To enhance the confidence of the results, the IB was calculated for 10 independent suspension realisations at each parameter condition. The mean IBs are presented in Fig. 3. Although the mean IB was also calculated for the *cellLocRange* = 0 case, it is not presented in the figure as it fluctuates greatly with volume fraction, with no apparent trend. This behaviour is due to the perfect lattice nature of these packs; as the volume fraction is varied, the uniform spacing of the cells will inevitably correspond to an ideally constructive or destructive interference condition for the pulse, shown in Fig. 1, used as the elemental scattered pulse from individual cells in the model.

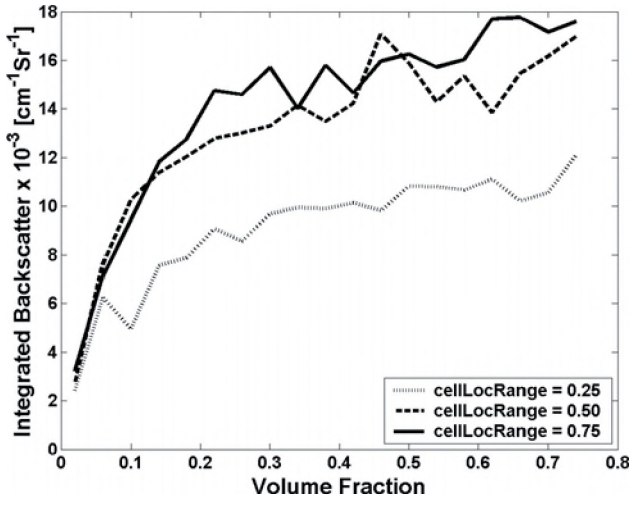

Fig. 3. The IB from simulated suspensions of cells with different degrees of position randomness (lower *cellLocRange* corresponds to more ordered, less random packing) for a wide range of volume fractions. Each IB curve represents the mean of 10 suspension realisations.

As expected, and previously documented (Hunt *et al.*, 1995), increasing the randomness of the scattering source positions increases the backscatter. In this model, because the randomisation parameter *cellLocRange* is relative to the volume fraction, one might expect the gap in mean IB between the *cellLocRange* = 0.25 and *cellLocRange* = 0.50 conditions to narrow for higher volume fractions, as the effective freedom of cell positions is reduced. However, this trend does not appear to exist. Nevertheless, for very low volume fractions, below 0.06, it is interesting to note that the degree of packing disorder does not appear to have a significant impact on backscatter.

The relation between IB and the fraction of the total observation volume occupied by cells, although appearing to plateau for high volume fractions, is generally positive. This is not what was seen with red blood cells (Shung *et al.*, 1984; Mo *et al.*, 1994), where both the measured and theoretical backscatter coefficients decreased for volume fractions (hematocrit) above 0.2. The authors of these studies concluded that this effect was due to increasing correlation between cell locations as the volume fraction increases; with more ordered packing, destructive interference between individual scattered pulses becomes more likely. Although the model presented here does not prevent cell overlap, which becomes possible with higher *cellLocRange* values, it is not likely the only reason that a decrease in backscatter is not observed. The red blood cell models (e.g. hard sphere, continuum) assume a simple scattering function, whereas it is clear from the single cell pulse in Fig. 1 that the scattering from a nucleated cell is not trivial, with possibly some resonant component.

It is anticipated that this model will become a useful tool when attempting to interpret experimentally measured backscatter from suspensions of different cell types.

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