MODELLING HIGH FREQUENCY ACOUSTIC BACKSCATTER RESPONSE FROM NON-NUCLEATED BIOLOGICAL SPECIMENS

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1. INTRODUCTION

It has been shown that high frequency ultrasound (20MHz - 60MHz) can be used to detect structural and physical changes in cell ensembles during apoptosis [1]. Apoptosis, or programmed cell death, was originally defined by Kerr *et al.* in 1972 [2] and is characterized by: nuclear condensation and DNA degradation, cytoplasm shrinkage, and fragmentation of the cell into membrane-bound bodies [3]. Ultrasonic backscatter from cell ensembles treated with the chemotherapeutic cisplatin (which induces apoptosis) increased by 9-13dB resulting in much brighter images in the areas the cells respond to the treatment. However, the mechanism that causes this increase in the backscatter is not known. Theoretical models of ultrasound scattering at the cellular level are needed in order to develop methods for using backscatter measurements to determine cell response to treatment. The development of such models requires an understanding of the various mechanical properties of the cell components.

Baddour *et al.* [4] performed successful measurement of high-frequency (10-65 MHz) backscatter frequency responses from a single eukaryotic cell. A recent study by the same group [5] showed that for prostate carcinoma (PC-3) cells whose nucleus to cell volume ratio equals 0.33, the backscatter response could be modeled as a fluid sphere. However, for human acute myeloid leukemia (OCI-AML-5) cells whose nucleus to cell volume ratio equals to 0.50, the backscatter response could not accurately be modeled as a fluid sphere.

This work attempts to investigate the response of some non-nucleated biological specimens by measuring the backscatter response from sea urchin oocytes and comparing it to theoretical predictions from a fluid sphere model to further provide evidence of their fluid-like nature.

2. METHODS

A very sparse suspension of oocytes from purple sea urchin were prepared in artificial seawater ($\rho \sim 1000\ Kg/m^3$, $c \sim 1500\ m/s$) at room temperature. These oocytes were selected because of their spherical shape and narrow size distribution (mean oocyte diameter 74 µm). A VisualSonics VS40B (VisualSonics Inc., Toronto, Ontario, Canada) ultrasound imaging device was used to acquired data using three transducers, with different resonant frequencies, f number, and focal lengths (20 MHz polyvinylidene fluoride: f2.35, 20 mm; 40 MHz polyvinylidene fluoride: f3, 9mm; 80 MHz lithium niobate: f3, 6 mm). These transducers were excited at 19, 40, and 55 MHz pulses, respectively. Only data from the -6-dB bandwidth of each transducer were used in the analysis which gave an overall bandwidth spanning 10-62 MHz. Ten independent acquisitions rf signals were performed using each transducer. The acquired rf lines were then thresholded by discarding all lines not containing any data greater than 90% of the maximum value found in all rf lines for a given transducer in order to remove empty rf lines and indirect oocyte hits. A Hamming window of width of 2 µs was applied to all remaining lines. In addition, visual inspection was performed to eliminate lines which exhibited abnormal pattern due to the presence of more than one oocyte in the resolution volume of the transducer. For each transducer, the remaining rf lines were superimposed, then averaged to obtain a single rf line corresponding to the average backscatter from a single oocyte. The backscatter transfer function ($\mathrm{BSTF}_{\mathrm{expr}}(\omega)$) was calculated as:

$$\mathrm{BSTF}_{\mathrm{expr}}(\omega) = \frac{R_{\mathrm{expr}}(\omega)}{R_{\mathrm{ref}}(\omega)}$$

where $R_{\mathrm{expr}}(\omega)$ is the Fourier transform of the average backscatter signal from a single oocyte. $R_{\mathrm{ref}}(\omega)$ is the Fourier transform of the reference signal (the reflection from a flat S_1O_2 crystal placed at the transducer focus in artificial sea water at room temperature). The values of the backscatter transfer function are shown in the form of plots expressed in relative decibels (dBr) to the backscatter intensity from the reference. The least squares analysis was used to determine the theoretical frequency responses that best agree with their corresponding experimental backscatter transfer functions. This was performed by minimizing R^2, the sum of the squares of the offsets of the theoretical responses from their corresponding experimental BSTFs. Theoretical frequency responses were calculated for a fluid sphere using the Faran scattering model [6] by the letting the Poisson's ratio approach 0.5.

3. RESULTS

Figure 1 shows the theoretical (Faran model: oocyte density=1168 Kg/m^3, speed of sound=1554 m/s) and measured backscatter frequency responses of a single sea urchin oocyte in sea water at room temperature subject to three incident pulses (19 MHz, 40 MHz, and 55 MHz). A contour plot of the least square fit coefficient, R^2, of experimental and theoretical backscatter transfer functions as a function of the oocyte density and speed of sound is shown in figure 2. Experimental data for the 20 MHz transducer (which generated the 19 MHz pulse) were not used in the calculation of R^2 due to the presence of ripples in the experimental backscatter frequency responses of the oocyte (see figure 1). Densities and speeds of sound for the oocyte ranged from $1100\ Kg/m^3$ to $1250\ Kg/m^3$ and from $1520\ m/s$ to $1580\ m/s$, respectively.

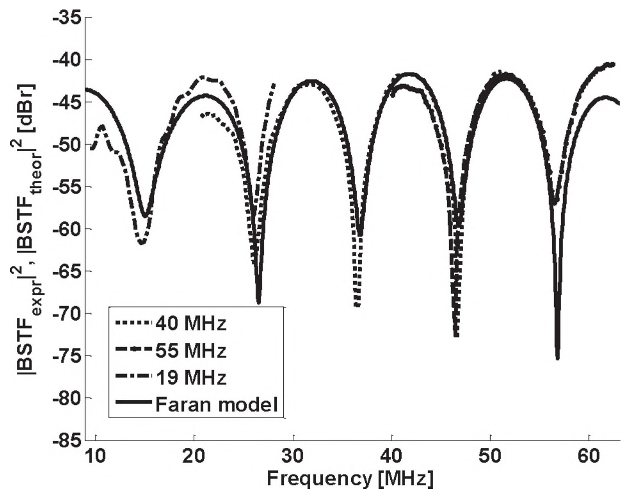

Fig. 1. Theoretical (Faran model) and measured backscatter frequency responses of single sea urchin oocytes in sea water at room temperature subject to three incident pulses: 19 MHz, 40 MHz, and 55 MHz.

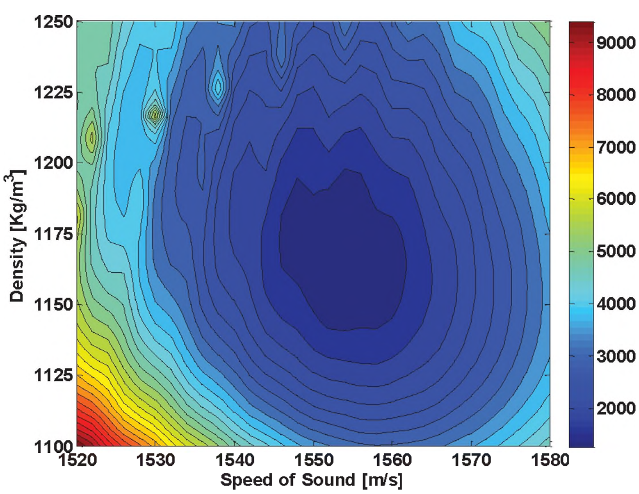

Fig. 2. Contour plot of the least square fit coefficient, R^2, of the experimental and theoretical backscatter transfer functions as a function of the sphere density and speed of sound.

4. DISCUSSION

The absence of the sharp resonance peaks in the frequency responses from a single sea urchin oocyte (figure 1) reveal the absence of detectable shear waves propagation inside the oocyte. This implies that oocytes behave more like fluid scatterers at high ultrasonic frequencies. For the 20 MHz transducer, the presence of ripples in the experimental backscatter transfer functions may be due to the transducer's low performance. This will be further investigated in future work. The least square fit analysis reveals that a density and speed of $1168\ Kg/m^3$ and $1554\ m/s$, respectively, provide the best fit of the theoretical frequency responses with their corresponding experimental backscatter transfer functions. A value of $1168\ Kg/m^3$ for the density is not surprising since sea urchin oocytes tend to settle at the bottom of the container when suspended in sea water. Similarly, the speed of sound in the sea urchin was found to be $1554\ m/s$. This value is very reasonable since it is close to that of biological tissue ($1540\ m/s$).

In conclusion, this study shows that the high frequency ultrasound backscatter from some non-nucleated biological specimens, such as sea urchin oocytes, are best modelled using the fluid sphere scattering model. Future work include the application of this methodology to investigate scattering from AML cells using a finite-element layered model, in which the cell is represented by a fluid shell (cytoplasm) surrounding an elastic sphere (nucleus).

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ACKNOWLEDGMENTS

This project was funded by the Canadian Institute of Health Research (grant #79447). This research was undertaken, in part, thanks to funding from the Canada Research Chairs Program awarded to Michael Kolios. Omar Falou is the recipient of an Ontario Graduate Scholarship. Funding to purchase the equipment was provided by the Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation and Ryerson University.