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● *Original Contribution*

HIGH-FREQUENCY ACOUSTIC IMPEDANCE IMAGING OF CANCER CELLS

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Abstract—Variations in the acoustic impedance throughout cells and tissue can be used to gain insight into cellular microstructures and the physiologic state of the cell. Ultrasound imaging can be used to create a map of the acoustic impedance, on which fluctuations can be used to help identify the dominant ultrasound scattering source in cells, providing information for ultrasound tissue characterization. The physiologic state of a cell can be inferred from the average acoustic impedance values, as many cellular physiologic changes are linked to an alteration in their mechanical properties. A recently proposed method, acoustic impedance imaging, has been used to measure the acoustic impedance maps of biological tissues, but the method has not been used to characterize individual cells. Using this method to image cells can result in more precise acoustic impedance maps of cells than obtained previously using time-resolved acoustic microscopy. We employed an acoustic microscope using a transducer with a center frequency of 375 MHz to calculate the acoustic impedance of normal (MCF-10 A) and cancerous (MCF-7) breast cells. The generated acoustic impedance maps and simulations suggest that the position of the nucleus with respect to the polystyrene substrate may have an effect on the measured acoustic impedance value of the cell. Fluorescence microscopy and confocal microscopy were used to correlate acoustic impedance images with the position of the nucleus within the cell. The average acoustic impedance statistically differed between normal and cancerous breast cells (1.636 ± 0.010 MRayl vs. 1.612 ± 0.006 MRayl), indicating that acoustic impedance could be used to differentiate between normal and cancerous cells. (E-mail: mkolios@ryerson.ca) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Acoustic impedance, Breast cancer cells, Acoustic impedance imaging, Acoustic microscopy.**

INTRODUCTION

Ultrasound wave interactions with homogeneous spherical or cylindrical objects are a well-understood phenomenon (Faran 1951). The acoustic impedance (in the case of a plane wave in an inviscid fluid medium) is equal to the product of density and speed of sound (Cobbold 2007). However, ultrasound wave interactions with cells and their complex microstructure are not as well understood (Mamou et al. 2008). Variations in acoustic impedance throughout the cell can alter ultrasound scattering interactions (Mamou et al. 2005, 2008), which can be used to detect anatomic and physiologic changes in tissues.

Changes in mechanical properties have been used to obtain information about the physiology and environment of cells (Suresh et al. 2005; Weiss et al. 2007). Mechanical properties are sensitive to changes in density, speed of sound or elastic properties of the cell at the length scale of the interrogating wavelength of ultrasound (Kolios 2009). Physiologic changes in the cell, including cell division, cell motility, cell adhesion, gene expression, signal transduction, apoptosis and the maturation of parasites in red blood cells, have been linked to changes in the elastic properties of the cell (Bao and Suresh 2003; Fuchs and Weber 1994; Ingber 2002; Weiss et al. 2007).

Acoustic impedance fluctuations can be used to acquire information about the tissue anatomic structures through calculation of the backscatter coefficient (BSC) (Mamou et al. 2005, 2008). The BSC is dependent on the relationship between an incident ultrasound wave of a particular wavelength, acoustic parameters (such as the density and speed of sound) and the size of the scattering object. The BSC has been used to characterize tissue microstructures in ocular, myocardial, liver and kidney tissues (Insana et al. 1992; Lizzi et al. 1983, 1987; Wear 1987). Additionally, maps of the spatial distribution of the BSC were used to monitor high-intensity focused ultrasound (HIFU)

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treatments (Kemmerer et al. 2010), detect abnormal lymph nodes (Mamou et al. 2011), differentiate between cancer tissues (Hruska et al. 2009; Oelze et al. 2004) and identify various forms of cell death (Kolios et al. 2002, 2003). In some cases, the BSC could not be used to identify breast cancers, although the optical images displayed a clear difference in the cellular microstructure (Oelze and Zachary 2006). Currently there is no consensus on the dominant ultrasonic scattering source of biological structures. Some studies support the hypothesis that the whole cell acts as a dominant scatterer source (Falou et al. 2010; Oelze and Zachary 2006). Other studies suggest that the nucleus is the main scatterer source when cells are embedded in tumors or surrounded by other cells (Czarnota et al. 1999; Kolios et al. 2003; Taggart et al. 2007). Thus, acoustic impedance fluctuations can be used to identify the dominant tissue scattering source, improving the diagnostic and monitoring capabilities of ultrasound. An understanding of the shape and mechanical properties of biological structures using acoustic impedance maps can provide information that can aid ultrasound tissue characterization.

Breast tumors are the most common malignant tumor in the world among women (Carkaci et al. 2011). Ultrasound can be used to detect these tumors; sonography is used to detect changes in mechanical properties (Carkaci et al. 2011; Mathis 2011). The detected changes in ultrasound contrast are not always due to tumor growth; therefore, biopsies are used for confirmation. A biopsy is an invasive, expensive and time-consuming procedure; a non-invasive method of determining malignancy has an impact on the diagnosis of breast tumor (Mamou et al. 2005; Tohno et al. 2009). An understanding the mechanical properties of normal and cancerous breast cells can be used to better analyze the ultrasound signals acquired from sonography and, in principle, increase its sensitivity in identifying tumors.

Hozumi et al. (2005) developed the acoustic impedance imaging method (AIIM), which has been used to create acoustic impedance maps of tissues. This method assumes that the dimensions of the scattering source are significantly larger than the ultrasound wavelength and the incident angle is normal to the surface of the tissue. Under these assumptions, the reflection coefficient caused by impedance mismatch between fluid–fluid interfaces can be simplified to

$$R = \frac{Z_2 - Z_1}{Z_2 + Z_1} \quad (1)$$

where Z_1 and Z_2 are the acoustic impedances of the first and second fluids, respectively. The AIIM uses a reference material with a known acoustic impedance to measure the impedance of the sample (Hozumi et al. 2005). The sample is attached to a solid substrate with known properties (*e.g.*, polystyrene). In the case of a solid–fluid or fluid–solid interface with a defined incident angle to the surface, the reflection coefficient equation must account for the formation of shear waves in the solid layer (Hozumi et al. 2007; Mayer 1965). Also, the presence of multiple impedance mismatches within a short range (of the order of the ultrasound wavelength) will have an impact on the measured acoustic impedance.

Many techniques have been developed to measure the mechanical properties of biological structures, such as atomic force microscopy (Radmacher et al. 1995), magnetic tweezers (Puig-De-Morales et al. 2001), optical tweezers (Ashkin and Dziedzic 1987), micropipette aspiration (Chapman 1982), shear-flow methods (Usami et al. 1993) and stretching devices (Wang et al. 2000). These techniques apply stress on the biological structures to measure its mechanical properties. Acoustic microscopy is desirable because of the minimal stress it applies to the sample and the relatively high resolution and speed (Kundu et al. 2000). The backscattered signal can be analyzed in the time domain or frequency domain (Kundu et al. 2000; Weiss et al. 2007). Time-domain analysis can be used to measure the speed of sound and calculate the acoustic impedance of the sample. The speed of sound is measured from the time shift between the sample and the reference, whereas the acoustic impedance is measured from the amplitude change between the sample and the reference (Strohm et al. 2010; Weiss et al. 2007). Frequency-domain analysis can be used to measure the density and speed of sound from the phase analysis or interpolation of the voltage-versus-frequency plot (Kundu et al. 1991, 2000; Zhao et al. 2012). The acoustic impedance of single cells has been measured using time-resolution techniques on HeLa and fibroblast cells (Briggs et al. 1993; Hildebrand and Rugar 1984; Weiss et al. 2007). The setups for these experiments create an angle between the transducer and the cell surface that was not accounted for because of the difficulty in determining this angle. The variation in the angle will affect the measured acoustic impedance values. In contrast, the AIIM is the only proposed method that can be used to measure acoustic impedance maps of single cells and accounts for the angle between the transducer and cell surface by changing the setup to create a 90° angle between the transducer and the cell surface.

The main objective of this study was to use high-frequency ultrasound to calculate the acoustic impedance maps of normal and cancerous breast cells using the AIIM. The acoustic impedance values are calibrated to account for the incident angle of the transducer and the