Observing cytoskeletal changes in cancer cells using high-frequency (10-100 MHz) ultrasonic spectroscopy

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Abstract

The cytoskeleton is pivotal to the biomechanical properties of cells. It therefore plays a crucial role in the behavior and progression of cancer. Cytoskeletal changes can enable cancer cells to become more mobile, thereby facilitating their infiltration into tissue or metastasis to other parts of the body. Cytoskeletal anomalies can also be associated with specific molecular subtypes of a cancer. For example, the more aggressive subtypes of breast cancer, such as basal-like and Her2+, have mutations that alter the protein regulation of the cytoskeleton. These subtypes may, therefore, be detectable via their effect on the cytoskeleton and cell biomechanics. The objective of this work was to determine if high-frequency (10-100 MHz) ultrasonic spectroscopy can detect chemically induced changes in the cytoskeleton of cancer cells. Cell cultures of a human pancreatic carcinoma cell line (panc-1) were grown in monolayers and then treated with sphingosylphosphorylcholine (SPC), a bioactive lipid that rearranges the keratin components of the cytoskeleton. Continuous pulse-echo measurements of the cultures were taken over a period of one hour. Computer simulations were performed to verify the results. The simulations modeled the ultrasonic spectra based on the internal structure of the cells using a multipole expansion method. The experimental spectra showed changes that were consistent with the simulated spectra and the optically observed changes in the keratin network. The results of this research demonstrate that high-frequency ultrasonic spectra are sensitive to cytoskeletal changes in cancer cells induced by SPC.

Keywords: High-frequency ultrasound, cytoskeleton, cell biomechanics
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1 Introduction

Cytoskeletal properties determine the biomechanical characteristics of cells and their role in many biomolecular processes. Examples include T-cell activation during the immune response and microtubule disintegration in Alzheimer’s disease. In breast cancer, cytoskeletal alterations are pivotal to tumorigenesis, the infiltration of tumor cells into tissue, and metastasis [1,2]. For example, changes in cell stiffness have been measured with laser-induced deformation during the tumorigenesis of normal human breast epithelial cells to malignant and metastatic cells [2]. Cytoskeletal structure and composition also varies as a function of the molecular subtype of breast cancer. Elevated protein expressions have been found in the more aggressive subtypes (Her2+ and basal) that are directly associated with the actin component of the cytoskeleton [3]. These proteins regulate the actin cytoskeleton and have the potential to alter the biomechanical properties, and thus aggressiveness and metastatic potential, of tumor cells. The proteins include thymosin β4 and β10 (involved in cytoskeletal binding), keratin type I cytoskeletal 19 (involved in metastatic progression of breast cancer), coactosin-like 1 (regulates actin cytoskeleton), and filamin A, alpha isoform 2 (anchors transmembrane proteins to actin cytoskeleton) [3].

Sphingosylphosphorylcholine (SPC) is a bioactive lipid that has been previously linked to the perinuclear reorganization of keratin filaments in Panc-1 cells. SPC is a bioactive lipid that is involved in cell migration and proliferation, and it is found in the high density lipoproteins (HDL) cells of the body. SPC has also been found at higher levels in the blood and malignant ascites of patients with cancer of the ovaries, and it is believed that SPC is involved in cancer metastasis [4]. SPC has previously been linked to the increase elasticity of Panc-1 cells through the perinuclear reorganization of keratin filaments [4].

There are many methods that have been used to measure the biomechanical properties of cells, including atomic force microscopy, micropipette-induced deformation, optical tweezers, and plate-induced shear force deformation. The disadvantages of these methods are that they are time-consuming and often complex to implement. A method is greatly needed for determining cell biomechanical properties that is simple, rapid, non-invasive, and capable of high-throughput measurements and real-time monitoring.

Ultrasound is a rapid, nondestructive, and straightforward method that reveals both the structure and mechanical properties of materials. This project explored the use of high-frequency (HF) ultrasound in the 10-100 MHz range for measuring SPC-induced cytoskeletal changes in Panc-1 cell cultures. The hypothesis for our research was that SPC-induced cytoskeletal changes would modify the biomechanical properties of the cells, and thus their acoustic scattering properties. These changes in scattering properties should be detectable by ultrasound having wavelengths close to the same order of size as the cells. Both computational and experimental studies were performed to test this hypothesis and interpret the results.
2 Methods

2.1 Computational Modeling

Computer models were used to determine whether changes in cell biomechanical properties would affect high-frequency ultrasonic signals from monolayer cultures. The models simulated HF ultrasonic backscattering from monolayers of spherical cells with spherical nuclei (Figure 1). The models employed multipole expansions of vector spherical wave functions, incident plane waves, and boundary condition solutions (continuity of stresses and displacements) to calculate the scattered wave field amplitudes at a virtual transducer located above the monolayer. Frequency dependent attenuation and the response function for a 50-MHz broadband transducer (60-MHz FWHM) were additionally incorporated into the models to simulate more realistic measurement conditions in growth media. Cells were simulated with a range of cell and nuclear diameters, as well as with a range of bulk and shear moduli for both the cytoplasm and nucleus. Bulk and shear moduli for the cytoplasm were estimated from ultrasonic measurements of a variety of tissues, including normal breast tissue, malignant breast tissue, and brain tissue. The simulation programs were written and executed in FORTRAN, and generated HF ultrasonic spectra in the 20-100 MHz band.

![Computational model of ultrasonic backscattering from spherical cells and nuclei.](image)

Figure 1: Computational model of ultrasonic backscattering from spherical cells and nuclei.

Figure 2a shows a simulated non-confluent cell monolayer constructed from a two-dimensional, hexagonal close-packed cell monolayer. Figure 2b displays the corresponding wave field displacements predicted by the scattering model. Figure 2c presents a plot of HF ultrasonic spectra from a simulated cell monolayer where the bulk modulus of the cytoplasm was varied from 2.1 to 2.5 GPa. The simulations reveal that changes in the cytoplasm mechanical properties, which result directly from cytoskeletal structure, can have significant effects on the position, amplitude, and shape of the HF spectral peaks.

2.2 Cell Culture

Panc-1 cells were purchased from American Type Culture Collection (ATCC) and cultured in DMEM with 10% Fetal Bovine Serum (FBS) during cell line maintenance. Cells were incubated at 37°C with humidified 95% air and 5% CO₂. Cell cultures were seeded at 2.5x10⁵ cells per well and tested 3-5 days after seeding. Cells were incubated in serum-free DMEM for approximately
6-8 hours before SPC treatment. SPC was purchased from Matreya and stored at -80°C until use. A 17.2 µM concentration of SPC was prepared in serum-free DMEM and added to cells immediately before testing with the HF ultrasound system.

**Figure 2:** (a) Computer model of cell monolayer. (b) Modeled ultrasonic wave fields in cell monolayer. (c) Modeled ultrasonic spectra as a function of cytoskeletal bulk modulus.

### 2.3 Ultrasound Method

An ultrasonic immersion transducer (Olympus NDT, V358-SU, 50 MHz, 0.635-cm dia. element) was submerged directly into the growth media of the cell culture (Figure 3a). An aluminum test fixture was used for placement of the cell culture plate directly below the transducer. A HF square-wave pulser-receiver (UTEX, UT340) was connected to the transducer and a digital oscilloscope (Agilent, DSOX3104A, 1 GHz, 4 analog channels) was connected to the pulser-receiver for pulse-echo measurements (Figure 3b) [5]. After an initial signal was located, a laptop personal computer with LabVIEW was used to record triplicate measurements every minute over a 60-minute period during incubation.

**Figure 3:** (a) Ultrasonic transducer immersed in growth media for acquiring cell culture measurement. (b) High-frequency ultrasound system.
2.4 Data Analysis

Figure 4 shows a representative waveform of the cell monolayer (Figure 4a) compared to a representative control waveform (Figure 4b). The control waveform is obtained by collecting a signal from a polystyrene cell culture plate well with no cells seeded in it. This well contains growth media only. For analysis purposes, a Mathcad computer program was used to isolate the cell culture waveform from the control waveform (Figure 5). Further analysis of the isolated cell waveform was performed using a Fourier transform to obtain the frequency spectrum.

![Figure 4: (a) Cell culture waveform with both cell and polystyrene well bottom reflection. (b) Control waveform with only polystyrene well bottom reflection.](image)

![Figure 5: Cell culture waveform showing polystyrene well bottom reflection (blue) overlaid with cell culture reflection (red).](image)

3 Results

Figure 6 displays cell waveform amplitudes for two Panc-1 cultures tested side by side, with Figure 6a representing the peak amplitude of the waveform, and Figure 6b displaying a color map...
of the entire cell waveform as a function of test time. Cultures A and B were first tested with HF ultrasound and serum-free DMEM to obtain Controls A and B in Figure 6. Cultures A and B were then treated with SPC; the data from these tests correlate to SPC A and SPC B in Figure 6. The waveform amplitude varied between 0.20-0.26 volts in both Control A and Control B; however, the waveform amplitude is fairly steady throughout the 60 minutes of testing (i.e., there are no sudden amplitude changes). In both SPC A and SPC B testing conditions, there is a large increase in waveform amplitude for the first 10-20 minutes of testing followed by a leveling off of waveform amplitude for the remaining treatment time.

Figure 6: Waveform amplitude data for SPC-treated Panc-1 cells.

Figure 7a shows the spectral peak frequencies over time for cultures A and B. Controls A and B show a steady peak frequency around 20 and 18 MHz, respectively, over the 60-minute test period. SPC A shows a steady peak frequency around 15 MHz over time. In contrast, SPC B displays a significant decrease in peak frequency during the first 15 minutes of testing, from 18 MHz to 14 MHz, followed by a leveling off of peak frequency to 13 MHz.

Figure 7b displays the observed peak frequency over time for a third cell culture test of Panc-1 cells with SPC. Control C shows a slowly and consistently increasing peak frequency over the 60-minute test period from 16 MHz to 20 MHz. In contrast, SPC C shows a significant decrease in peak frequency during the first 15 minutes of testing, from 22 MHz to 10 MHz. The peak frequency then stabilizes for the remainder of the testing period to around 12.5 MHz.

The waveform amplitude results from SPC A and SPC B, and the spectral peak results from SPC B and SPC C, confirm that chemical-induced modifications to the cytoskeletal architecture can be measured and monitored in real time with HF ultrasound. The tests show that there are decreases in both the waveform amplitudes and spectral peak frequencies upon treatment of Panc-1 cells with SPC. The decrease in peak frequency substantiates the predicted changes in peak frequency observed previously in the generated computer simulations.
4 Conclusions

In looking at both the waveform amplitude data and the peak frequency data, there are noticeable differences between control cells and SPC treated cells. The initial large waveform amplitudes and high peak frequencies observed in the first 10-20 minutes of SPC treatment could be attributed to the time it takes for keratin reorganization into a perinuclear formation as previously observed [4]. As predicted by the computer simulations generated, it was expected that the peak frequency would decrease in correlation with an increase in the bulk modulus of the cell. Upon treatment with SPC, there was an observed decrease in the peak frequency of the Panc-1 cells tested. This indicates that the HF ultrasound is detecting the condensation of the keratin filaments around the nucleus, which increases the bulk modulus of the inner region of the cytoplasm. Other methods for measuring the biomechanical properties of cells primarily measure the outer region of the cytoplasm, and have thus measured a decrease in the bulk modulus for SPC-treated Panc-1 cells since the keratin filaments are depleted in the cytoplasm’s outer shell.

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References


