Optical Coherence Tomography detection of shear wave propagation in MCF7 cell modules

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In this work, we explored the potential of measuring shear wave propagation using Optical Coherence Elastography (OCE) in MCF7 cell modules (comprised of MCF7 cells and collagen) and based on a swept-source optical coherence tomography (OCT) system. Shear waves were generated using a piezoelectric transducer transmitting sine-wave bursts of 400 μs, synchronized with an OCT swept source wavelength sweep imaging system. Acoustic radiation force was applied to the MCF7 cell constructs. Differential OCT phase maps, measured with and without the acoustic radiation force, demonstrate microscopic displacement generated by shear wave propagation in these modules. The OCT phase maps are acquired with a swept-source OCT (SS-OCT) system. We also calculated the tissue mechanical properties based on the propagating shear waves in the MCF7 + collagen phantoms using the Acoustic Radiation Force (ARF) of an ultrasound transducer, and measured the shear wave speed with the OCT phase maps. This method lays the foundation for future studies of mechanical property measurements of breast cancer structures, with applications in the study of breast cancer pathologies.

Keywords: Shear wave, Elastography, Optical Coherence Elastography, Acoustic Radiation Force (ARF), mechanical properties, MCF7 cells, Collagen.

1. Introduction

Elastography is a method in which stiffness or strain images of tissue are generated to characterize biomechanical properties [1]. An imaging modality is used to image the deformation behavior of the biomaterial under a static or dynamic loading and generate so-called elastograms . Elastograms contain information about local variations of the stiffness inside a region of interest and may provide information to aid in the identification of biomaterial defects or tissue properties. The shear modulus of biomaterials in particular is thought to be highly sensitive to variations in biomaterial structure and composition. Different imaging modalities can be used to measure displacements and estimate the resulting mechanical properties such as ultrasound or magnetic resonance imaging (MRI) [2]. The main drawbacks of MRI is that it is technologically complex and expensive. Moreover, both MRI and ultrasound do not have sufficient resolution to detect small scale and subtle elastic variations in biomaterials and tissues (such as in tissue engineered

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modules and microscopic breast cancer). Optical Coherence Tomography (OCT) is an optical tomographic imaging technique that shares many similarities to ultrasound despite using light. OCT has several advantages over other imaging modalities, primarily related to its inherently high resolution and motion sensitivity, which allows for the identification of micron-sized morphological tissue structures and highly localized strains [3-5]. The technology is flexible and inexpensive. Optical coherence elastography (OCE) measures tissue displacement using OCT and benefits from the high spatial and temporal resolution of this imaging technology. OCE is a relatively new elastography technology used to measure biomechanical properties of soft tissue [3]. During OCE, the tissue can be excited internally or externally and statically or dynamically [4]. Methods of creating dynamic compressions include acoustic radiation force (ARF) and low-frequency vibration [5].

The shear-wave propagation speed and attenuation depend on the mechanical properties of the tissue [6, 7]. Typical values for the speed of shear waves in tissue are on the order of a 1-10 m/s, and the attenuation coefficient of soft tissues are two or three orders of magnitude greater than that of compressional waves. Due to the high attenuation of shear waves, the shear wave is generated only within a very limited area of tissue [7]. The frequency content of the shear wave will be determined primarily by the beam width of the ultrasound beam and is not dependent on the time duration of the excitation, unless the excitation duration approaches the natural time constants of soft tissues.

2. Method and materials

Optical Coherence Tomography (OCT) provides imaging with histological resolution, which allows for the identification of micron sized tissue structures. Optical coherence elastography (OCE) can measure tissue displacement with the high resolution of OCT to generate high-resolution stiffness maps. In this work, we show we can measure shear wave propagation in MCF7 cell modules using OCT as an imaging modality and measure mechanical properties of MCF7 cells.

To achieve this, the acoustic radiation force produced by focused ultrasound beams was used to produce the shear waves. The ARF (internal mechanical excitation) was applied to the cell modules using a 20 MHz, circular, piezoelectric transducer element (PZT, f-number 2.35) transmitting sine-wave bursts of 400 μs. The internal displacements induced by the shear waves were detected using optical coherence tomography and phase sensitive motion algorithms. A Thorlabs SS-OCT system was used in this study. The laser had a center wavelength of a 1310 nm, a bandwidth of ~110 nm, and an A-scan rate of 8 kHz. The lateral resolution was approximately 13 μm in the samples. Using this technique, mechanical properties of the MCF7+collagen can be measured.

Module protocol: Collagen modules were prepared as previously described [8]. In brief, collagen at 3.1mg/mL (Advanced BioMatrix, San Diego), was mixed with 10x minimum essential media (Gibco, from powder, made up to 10 times normal concentration, 128μL/mL of collagen) , and neutralized with 0.8M $NaHCO_3$ (60μL/mL collagen) and kept on ice. MCF7cells, grown in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS) and 0.1% insulin at

37°C and 5% CO_2, were trypsinized, counted, and then resuspended at 4 x 10^6/mL in the collagen solution. The collagen was dispensed into tubing with an inner diameter of 1.5mm, and incubated at 37°C for 1 - 2 hours. Once the collagen solidified, tubing was hand-cut to 0.5 to 1cm lengths, deposited into a 50mL tube, and DMEM + 10% FBS was added. The 50 mL tubes were then agitated to release the modules from the tubing, the media was removed and the modules were transferred to a new 50mL tube. Modules were centrifuged for ~5min at 125g to form a pellet. The majority of the supernatant was removed, and the modules were transferred to the gel for OCE and shear wave imaging. Experiments were done in duplicate.

Bright field images of the modules were obtained on an Olympus CKX41, with a QImaging Retiga 2000R camera and QCapture software. Uneven cell dispersion, visible in Figure 1, occurs due to the cell sedimentation within the collagen as it sets.

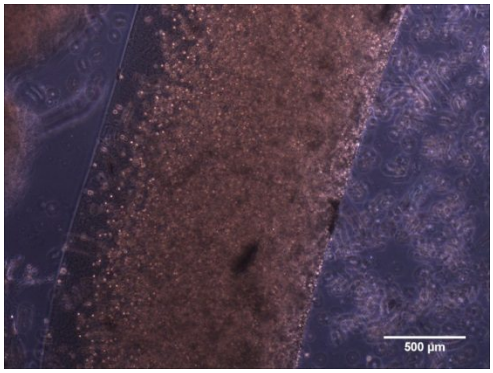

Fig. 1. Bright field images of the modules were obtained on an Olympus CKX41, with a QImaging Retiga 2000R camera and QCapture software illustrating a high cellular density in the module.

We used the same experimental set-up as in our previous work [9]. The OCT signals from the samples were used for the measurement of the shear wave speed and mechanical properties of MCF7 cells. The US "push" transducer was synchronized with the OCT imaging system. The phase analysis was applied to B-mode and M-mode OCT images, which were obtained while the US transducer was generating the "push" in the phantom. A fast Fourier transform was performed on the OCT data, and phase maps of the phantom under US loading were generated and are directly related to the ARF induced displacement in the phantom.

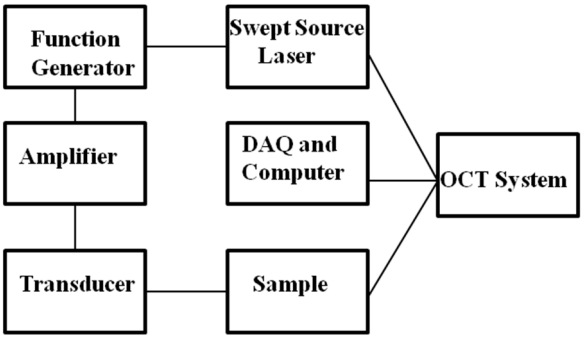

Fig. 2. A schematic diagram of the ARF-OCE experimental setup. The setup consisted of the existing SS-OCT system, a MCF7+ Collagen tissue construct phantom, a focused transducer (20 MHz, f-number 2.35), an amplifier and a function generator (Agilent 33250A 80 MHz, Function / Arbitrary Waveform Generator) synchronized with the SS-OCT system.

3. Results

OCT images of the MCF7 cell modules were taken with the SS-OCT system and B- mode phase maps were obtained. These data provide information that is required to calculate the distance between two measurement points and the phase shift between these two locations. These parameters (Δr and $\Delta\varphi$) are required to calculate the shear wave speed. As described in more detail in previous work [0], using Δr and $\Delta\varphi$ we can calculate the shear modulus and Young modulus. After the OCT imaging session, MCF7 cell samples were fixed in formalin for 48 hours. Hematoxylin and eosin (H&E) stains were performed, followed by digital pathology slide scanning (ImageScope, Aperio, Vista, CA) for quantitative measurement of MCF7 cells and collagen.

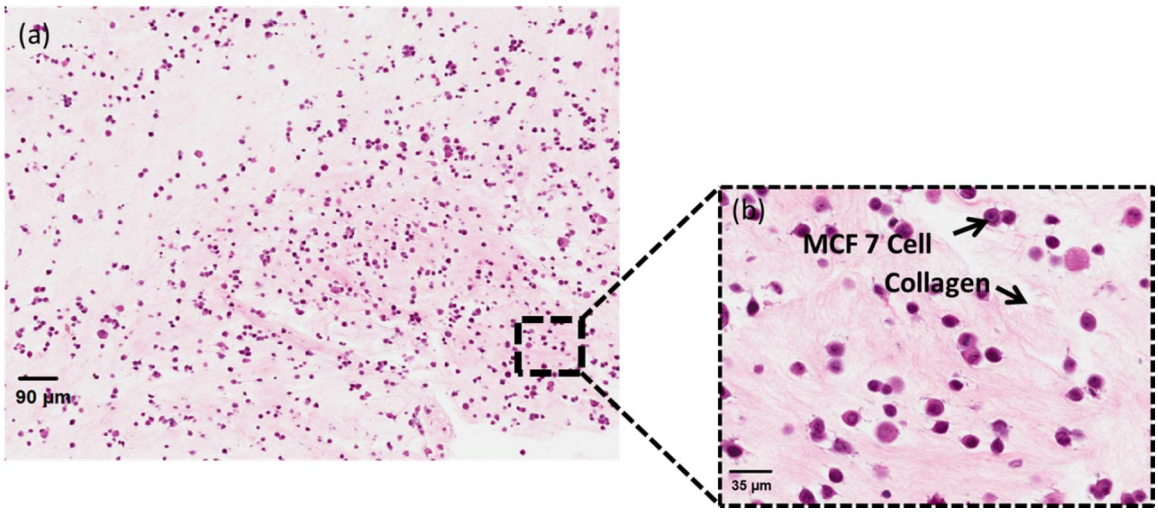

Fig 3: (a) H&E histology demonstrating the MCF7 cells and collagen and (b) higher magnification image taken from highlighted inset in (a). The scale bar in (a) in 90 µm and 35 µm in (b).

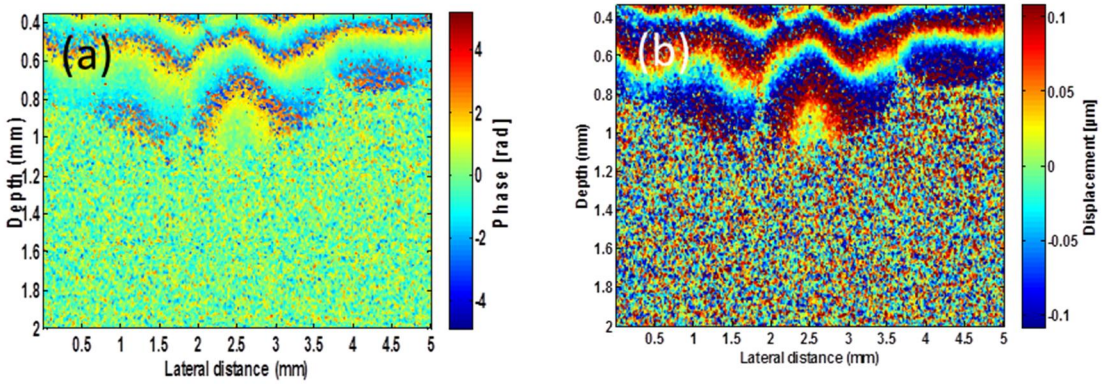

Fig. 4. (a) The B-mode phase map of the MCF7 was used to measure Δr and $\Delta\varphi$ for the calculation of the shear wave speed. The color scale represents the change of the phase value (radians). **(b)** Displacement snapshot of the shear wave propagating through the MCF 7 module.

The displacement z is calculated from the measured phase as $z = \lambda_0\Delta\phi/4\pi n$ [10] where λ_0 is the center wavelength of the OCT system and n is the sample refractive index. In figure 4 the x-axis is lateral distance within the phantom and y axis is depth. The color represents the displacement calculated from the phase maps.The shear modulus and young modulus were measured to be 7.1±0.1 kPa and 21.5±0.3 kPa respectively.

4. Discussion

OCT provides greater spatial and phase resolution than previous methods that have been used for the study of the deformation of tissue and biomaterials. This allows for the detection of small deformations in the MCF 7 cell modules that may be important to the measurement of tissue mechanical properties. The spatial resolution of mechanical property maps will depend on whether reliable phase difference measurements between two locations can be made with SW-OCE. These maps have much better spatial resolution compared to the shear wave wavelength, and therefore it is expected the method will allow the measurement of mechanical properties at micron resolution.

The OCT phase maps were acquired with a swept-source OCT (SS-OCT) system. Although SS-OCT systems typically have higher phase noise than SD-OCT systems, especially at high A-scan rates, the phase noise of the relatively low speed SS-OCT (8kHz bi-directional) used in these experiments was sufficient to measure phase changes induced by shear wave propagation. The OCT system is very sensitive as even very small vibrations or electronics device noise will effect on the phase stability. To minimize this effect, the OCT imaging system was placed on a vibration isolation optical table. Future work will focus on repeating the experiment with different collagen concentrations and the examination of inhomogenous phantoms with localized changes in shear modulus properties. OCT can be used to image shear wave propagation in MCF 7 cells and collagen with high resolution and will be used to measure the properties of inhomogeneous phantoms.

In summary, we have demonstrated a SW-OCE technique that uses ARF for mechanical excitation of MCF7 cell modules to measure shear wave propagation. The mechanical excitation produces motions within the sample that can be used for the estimation of mechanical properties using SW-OCE. By providing high-resolution maps of the mechanical properties of tissue, we hope that this technology can be used to identify changes in the mechanical properties of diseased tissue such as breast cancer.

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