Two-dimensional (2D) dynamic vibration optical coherence elastography (DV-OCE) for evaluating mechanical properties: a potential application in tissue engineering

Hsiao-Chuan Liu, Piotr Kijanka, and Matthew W. Urban

History

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Abstract

Mechanical properties in tissues are an important indicator because they are associated with disease states. One of the well-known excitation sources in optical coherence elastography (OCE) to determine mechanical properties is acoustic radiation force (ARF); however, a complicated focusing alignment cannot be avoided. Another excitation source is a piezoelectric (PZT) stack to obtain strain images via compression, which can affect the intrinsic mechanical properties of tissues in tissue engineering. In this study, we report a new technique called two-dimensional (2D) dynamic vibration OCE (DV-OCE) to evaluate 2D wave velocities without tedious focusing alignment procedures and is a non-contact method with respect to the samples. The three-dimensional (3D) Fourier transform was utilized to transfer the traveling waves (x, y, t) into 3D k-space (k_x, k_y, f). A spatial 2D wavenumber filter and multi-angle directional filter were employed to decompose the waves with omni-directional components into four individual traveling directions. The 2D local wave velocity algorithm was used to calculate a 2D wave velocity map. Six materials, two homogeneous phantoms with 10 mm thickness, two homogeneous phantoms with 2 mm thickness, one heterogeneous phantom with 2 mm diameter inclusion and an *ex vivo* porcine kidney, were examined in this study. In addition, the ARF-OCE was used to evaluate wave velocities for comparison. Numerical simulations were performed to validate the proposed 2D dynamic vibration OCE technique. We demonstrate that the experimental results were in a good agreement with the results from ARF-OCE (transient OCE) and numerical simulations. Our proposed 2D dynamic vibration OCE could potentially pave the way for mechanical evaluation in tissue engineering and for laboratory translation with easy-to-setup and contactless advantages.

1. Introduction

Tissue mechanics have been comprehensively investigated for the past two decades with elastography techniques because tissue mechanical properties are significantly associated with disease states. For example, fibrosis and cancer cause prominent changes of mechanical properties in tissues [1]. The changes of tissue stiffness correlate with extracellular matrix (ECM) fibers interacting with fibroblast proliferation and differentiation [2]. In addition, mechanical properties of tissues can be altered by cells themselves. Those cells dominate the machinery of tissues by their mechanical forces such as stiffness of cytoskeleton and their responses to surrounding microenvironment. In the case of cancer, solid stress, caused by growing tumors in a constrained physical volume, will increase abnormal cell proliferation. The abnormal proliferation compresses healthy surrounding tissue and eventually increases the stiffness of malignant lesions [3]. Therefore, mechanical properties of tissues are an important indicator to better understand various disease states and physiological conditions [4].

Atomic force microscopy (AFM) is a powerful tool for the quantification of mechanical properties of soft tissues and cells; however, it includes the risk that samples are regionally damaged by the compression from a scanning cantilever, and has high cost and involves time-consuming operation [5,6]. Wave propagation has been extensively used to evaluate biomechanical properties of soft tissues by using ultrasound shear wave elastography (SWE) [7–11] and magnetic resonance elastography (MRE) [12,13] in clinical applications. Generally, the spatial scales of SWE and MRE images are in the ranges of millimeters to centimeters such that it constrains investigations to macroscopic levels with an organ-sized field-of-view (FOV) [4,14]. Optical coherence tomography (OCT) is an alternative modality used to image tissue displacements on a micron-scale [14]. A number of advantages of OCT imaging include it being noninvasive, noncontact, fast, having high spatial resolution, and being sensitive to the topology of a surface. OCT-based elastography, named optical coherence elastography (OCE), was first proposed by J. Schmitt in 1998 to assess mechanical properties of the muscle and fat layers in a porcine meat sample and skin tissue by using compressive stress [15]. Numerous reports have shown the capability of OCE equipped with various excitation tools to generate shear wave or surface wave propagation in two-dimensional (2D) space for characterizing mechanical properties of tissues [16]. So far, it has been widely used for investigation of mechanical properties in biological tissues and biological fluids during the last two decades [16–19]. In wave-based OCE techniques, the spatial resolution closely depends on excitation frequencies, pulse duration for transient excitation and mechanical properties of samples (e.g., stiff or soft).

Many OCE experiments involve some form of contact method or are *ex-vivo* studies, i.e., taking a non-living tissue sample for measurement. The excitation tools either directly contact samples or contact containers used to hold samples, like a Petri dish or glass plate. For transient excitations with a contact method, Li, et al., used a piezo-vibrator contacting at approximately a 45° angle over a sample surface to measure surface waves and determine the elastic properties in skin, chicken breast and heterogeneous phantoms [20,21]. In a non-contact method, Ambroziński, et al., utilized a home-made air-coupled piezoelectric transducer to generate an air puff-based acoustic radiation force on an *ex vivo* porcine cornea for evaluating wave propagation and phase velocities [22]. A similar study was also conducted by S. Wang and K. V. Larin to assess the biomechanics of *ex vivo* rabbit cornea by using air-puffs generated via an air-gate controller [23]. Z. Jin et al. also used air-coupled ultrasound to measure 3D corneal elasticity *in-vivo* in a 3D manner [24]. A needle tip as a contact-based loading was reported for breast tissue characterizations [25,26]. Acoustic radiation force (ARF) is a reliable excitation method broadly used to evaluate mechanical properties of biological tissues and biological fluids such as carotid artery [27], porcine blood and plasma [18], cornea [28–30], retinal tissue [31] and crystalline lens [32–34].

However, using transient excitation methods in OCE can increase the difficulty in a translational setting for tissue engineering applications due to the need for complicated alignment and smaller samples. Besides, transient excitations can result in generating undesired reflected waves from rigid boundaries, and could increase the difficulty to evaluate correctly estimate wave velocities and affect the evaluation of mechanical properties [35]. Harmonic OCE has been reported that utilize waves of a fixed frequency in confined media. F. Zvietcovich, et al., used two vibrators with 400 Hz and 400.4 Hz harmonic frequencies respectively as excitation signals for generating waves in a heterogeneous phantom to evaluate shear wave velocities and mechanical measurements [36]. P. Meemon, et al., also used a similar system to distinguish two components of a phantom based on crawling waves [37]. This approach is limited by the sample size needed to allow waves to interfere, which may be relatively large compared with samples used for tissue engineering. Another approach took advantage of reverberant wave behavior to estimate the elasticity of individual corneal layers [38]; however, a customized multi-pronged excitation ring is required and contact with tissue cannot be avoided. On the other hand, those method could be difficultly implemented for tissue engineering applications due to samples usually consisted of hydrogel-tissue hybrid placed in a limited culture space (35 mm diameter wells as an example).

In this study, we report a new concept called 2D dynamic vibration OCE (DV-OCE) technique to evaluate mechanical properties of the samples in a confined environment to mimic the situation for tissue engineering applications. This proposed method is non-contact with respect to the sample and avoids a tedious excitation alignment process. This method could potentially be used for tissue engineering applications to measure mechanical properties of tissues growing in culture. A coil-based vibrator with harmonic excitation was used to generate traveling waves in two homogeneous materials of different thicknesses (7% and 14% gelatin, 10 and 2 mm thickness), a heterogeneous material and *ex vivo* porcine kidney tissue. The samples in 35 mm diameter Petri dishes were simply placed on the surface of vibrator without complicated excitation focusing alignments. A customized 3D OCT scan pattern was used to create 4D wave propagations (*x, y, z, t*). In addition, 2D wavenumber filter, multi-angle directional filter and 2D local wave velocity algorithm were implemented to determine wave velocities. Transient ARF-OCE was utilized to compare with our new 2D dynamic vibration OCE technique. Numerical simulations in the two homogeneous materials were performed to validate the experimental results. Our proposed 2D DV-OCE technique is robust in determining the wave velocities in the homogenous phantoms, the heterogenous phantom and the biological tissue without tedious focus alignment and contactless. The proposed method is a promising technique that could be applied for tissue engineering applications and laboratory translation under *easy-to-setup* procedures [1].

2. Materials and methods

2.1. Fabrication of homogeneous phantoms and a heterogeneous phantom

A total of five phantoms were fabricated for this study. Four phantoms were homogeneous phantoms and one is a heterogeneous phantom. The two gelatin concentrations, 7% v/v and 14% v/v, with 10 mm thickness were fabricated by using gelatin powder (gel strength 300 type A, G2500-1KG, Sigma-Aldrich, St. Louis, MO, USA) to make the homogeneous phantoms and 1 g of titanium dioxide (TiO_2, ReagentPlus ≥ 99%, Sigma-Aldrich, St. Louis, MO, USA) was used to provide optical scatterers. To provide cases that may be similar to those in tissue engineering applications, we created 7% v/v and 14% v/v homogeneous phantoms with 2 mm thickness as the advanced cases. A total volume of 100 mL tap water in a 500 mL beaker was heated to approximate 70 °C. The 14% v/v gelatin powders and 1 g TiO_2 were added with stirring to the beaker for approximately five minutes to homogenize the solution. The mixed solution was placed in a de-gassing chamber to remove small bubbles in the fluid.

After de-gassing, the mixed gelatin solution was poured into two regular 35 mm diameter × 10 mm height Petri dishes (for 10 mm and 2 mm thickness) for DV-OCE experiments and a custom 85 mm diameter × 10 mm height Petri dish with Mylar film bottom for ARF-OCE experiments to obtain the comparison results. The ARF has a small attenuation due to only 100 µm thickness of the Mylar film, which can be considered acoustically transparent [5,39,40]. The Petri dishes were transferred to a 4 °C refrigerator for congealing. The above procedure was repeated for the 7% v/v homogeneous gelatin phantom. For the fabrication of the heterogeneous phantom, the same 14% v/v gelatin solution was poured into another regular 35 mm diameter × 10 mm height Petri dish with a 2 mm diameter pillar placed in the middle of the Petri dish to create a small circular well for a 2 mm diameter inclusion. The pillar was removed once the 14% v/v gelatin solution was completely congealed, and the 7% v/v gelatin solution, following the above fabrication description, was poured into the small circular well. The heterogeneous phantom had an approximate 10 mm thickness.

2.2. Porcine kidney tissue preparation

A porcine kidney was excised immediately after sacrifice and frozen. The kidney was thawed and placed in saline solution at room temperature. The kidney was sliced in half in the long dimension and reflected. A slice of renal cortex with the approximate dimensions of 20 × 20 × 4 mm was cut for the experiment.

2.3. Three-dimensional (3D) numerical models

In order to validate our method, numerical simulations were performed using a finite element method. An explicit solver was used to solve the differential elastic wave equations in OnScale (OnScale, Redwood City, California). Two three-dimensional models with elastic material properties were examined. The models, consisting of 2,899,232 grid elements and 2,962,971 grid nodes, were used to create simulated phantoms with 35 mm diameter × 10 mm thickness. To reduce the computational burden and solution time, the models were modelled as axisymmetric models in two in-plane axes. Figure 1(a) presents the top and front views of the simulated phantom with specified the type and locations of the boundary conditions. The axes of x and y are symmetry. By assuming that the Petri dish is infinitely stiffer than the tested samples, we can use a fixed displacement boundary condition applied at the bottom and sides of the numerical phantoms. The mechanical transient analyses were performed using an explicit solver. The simulation time step is 0.0523 μs.