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Dynamic and quasi-static mechanical testing for characterization of the viscoelastic properties of human uterine tissue

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ABSTRACT

Ultrasound elastography is envisioned as an optional modality to augment standard ultrasound B-mode imaging and is a promising technique to aid in detecting uterine masses which cause abnormal uterine bleeding in both pre- and post-menopausal women. In order to determine the effectiveness of strain imaging, mechanical testing to establish the elastic contrast between normal uterine tissue and stiffer masses such as leiomyomas (fibroids) and between softer pathologies such as uterine cancer and adenomyosis has to be performed. In this paper, we evaluate the stiffness of normal uterine tissue, leiomyomas, and endometrial cancers using a EnduraTEC ElectroForce (ELF) system. We quantify the viscoelastic characteristics of uterine tissue and associated pathologies globally by using two mechanical testing approaches, namely a dynamic and a quasi-static (ramp testing) approach. For dynamic testing, 21 samples obtained from 18 patients were tested. The testing frequencies were set to 1, 10, 20, and 30 Hz. We also report on stiffness variations with pre-compression from 1% to 6% for testing at 2%, 3%, and 4% strain amplitude. Our results show that human uterine tissue stiffness is both dependent on percent pre-compression and testing frequencies. For ramp testing, 20 samples obtained from 14 patients were used. A constant strain rate of 0.1% was applied and comparable results to dynamic testing were obtained. The mean modulus contrast at 2% amplitude between normal uterine tissue (the background) and leiomyomas was 2.29 and 2.17, and between the background and cancer was 0.47 and 0.39 for dynamic and ramp testing, respectively.

1. Introduction

Ultrasound elastography has become a popular imaging technique for mapping tissue stiffness (Ophir et al., 1991). Tissue deformation for elastography is divided into two main groups: quasi-static and dynamic (Fatemi and Greenleaf, 2002). In quasi-static methods, tissue is compressed slowly and at least two frames of data, before and after the deformation (Ophir et al., 1999), or a data loop during the deformation are acquired (Varghese, 2009). Local displacements are estimated by comparing these data frames over small gated regions. Deformations can be applied using the transducer itself, either freehand or with mechanical devices. Strain computed from the displacement gradient is related to the stress distribution, if available, and resulting Lamé parameters calculated *via* elasticity equations (Sarvazyan et al., 2011). However, the stress distribution is generally not available and modulus reconstruction is performed by solving the inverse problem (Barbone and Bamber, 2002). In many clinical applications, strain distributions are used to determine tissue stiffness as a qualitative surrogate marker of elasticity; *i.e.*, low strain indicates high stiffness while large strain indicates a low stiffness region.

Mechanical testing has been used to quantify differences in elastic moduli and spatial distribution of Young's modulus for different tissue types (Krouskop et al., 1998; Mazza et al., 2007, DeWall et al., 2012). Detection and characterization of uterine masses causing abnormal uterine bleeding have been evaluated with elastography (Hobson et al., 2007; Omari et al., 2012), and its role for differential diagnosis of endometrial pathologies investigated by several research groups. Significant differences in endometrial stiffness have been reported for patients with

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atrophic endometrium confirmed with pathology (Preis et al., 2011). A pilot study using transvaginal real-time ultrasound elastography confirmed benign etiology based on the presence of an endometrial strip in the elastogram (Neale et al., 2011). To determine effectiveness of ultrasound elastography for differentiation of abnormal uterine bleeding etiology, the elastic or modulus contrast between normal uterine tissue and stiffer masses such as leiomyomas and between softer pathologies such as uterine cancer and adenomyosis is essential (Omari et al., 2012).

Human cervical and uterine tissue studies to quantify elastic and viscoelastic properties were conducted in our laboratory. Kiss et al. (2006) measured the complex modulus in *ex-vivo* cervical and uterine hysterectomy samples using dynamic testing. Small compressions, 1–2%, were applied over a wide frequency range spanning 0.1–100 Hz. Modulus values for cervical and uterine tissue increased monotonically from approximately 30 kPa to 90 kPa with an increase in testing frequency. Leiomyomas exhibited modulus values that ranged from 60 to 220 kPa.

Bauer et al. (2007) utilized an aspiration device for *in-vivo* cervical evaluations, to evaluate physiological and biomechanical changes through gestation for detecting pregnant women at risk of cervical incompetence. For *in-vivo* studies their stiffness parameter values varied from 0.065 to 0.315 bar/mm, while softening parameter values ranged from 0.05 to 0.19. *Ex-vivo* testing results ranged from 0.11 to 0.29.

Myers et al. (2008) performed ramp loading tests on cervical ring sections under three different testing modes: load–unload cycle, unconfined ramp-relaxation, and confined ramp-relaxation. Ramp testing is a quasi-static approach which subjects the sample to a constant strain rate over a large applied deformation, with the stress and strain measured continuously. Each specimen was first loaded under unconfined compression to a 15% axial strain and unloaded to 0% strain at a constant strain rate of 0.1% per second over three cycles. Their results indicated that cervical stroma has a nonlinear time-dependent stress response with varying degrees of conditioning and hysteresis depending on its obstetric background. Cervical tissue obtained from women who were never pregnant was significantly stiffer than women who underwent a pregnancy.

DeWall et al. (2010) quantified viscoelastic properties of normal human cervix through a range of pre-compressions (1–6%), compression amplitudes (2%, 3%, 4%), and testing frequencies (1, 10, 20, 30 Hz). This study revealed lower modulus values, by an order of 10, than those previously reported by Kiss et al. (2006). The storage modulus increased monotonically from approximately 4.7 kPa to 6.3 kPa over the pre-compression range of 1–6% at a testing frequency of 1 Hz. The material's damping ($\tan\delta$) remained fairly constant ($\sim$0.35) over this range. However, with an increase in the mechanical testing frequency both the storage modulus and damping increased (DeWall et al., 2010; Kiss et al., 2006).

In this paper, mechanical testing methods for global stiffness measurements of human uterine tissue are described. We compare results obtained using two mechanical testing techniques namely dynamic and ramp testing of normal uterine tissue, leiomyomas, and carcinoma.

2. Mechanical testing of human uterine tissue

Soft tissue behavior has been characterized to be anisotropic, viscoelastic and nonlinear. However, under certain simplifying assumptions, such as low strain and rapid load application, soft tissue can be assumed to be linear, elastic, and isotropic (Krouskop et al., 1998; Wells and Liang, 2011). Uterine and cervical tissues are viscoelastic. Transient properties such as creep and relaxation are illustrated in Fig. 1 to depict differences between elastic and viscoelastic materials.

Characterization of biological tissue stiffness can be performed globally or locally (Omari, 2014; DeWall et al., 2012). In order to characterize linear viscoelastic properties, the complex Young's modulus (E^*) is generally estimated. In this study, both dynamic and ramp loading tests using an EnduraTEC ElectroForce (ELF) (Bose Corporation, ElectroForce Systems Group, Eden Prairie, MN, USA) system, shown in Fig. 2, were performed for global stiffness estimation of uterine specimens. Normal, fibroid, and cancerous uterine specimens were obtained from UW Hospital and Clinics (Madison, WI) pathology lab following a hysterectomy procedure on patients. The protocol for sample acquisition was approved by the University of Wisconsin Institutional Review Board (IRB). Patient consent was obtained prior to acquisition of excised samples.

Samples were transported to the elastography lab in the Wisconsin Institute for Medical Research (WIMR), in a small container immersed in isotonic saline solution. Samples were kept refrigerated prior to testing, and were mechanically tested within a few hours of excision. Samples were brought to room temperature, cut to approximately a cubic centimeter in dimension, measured

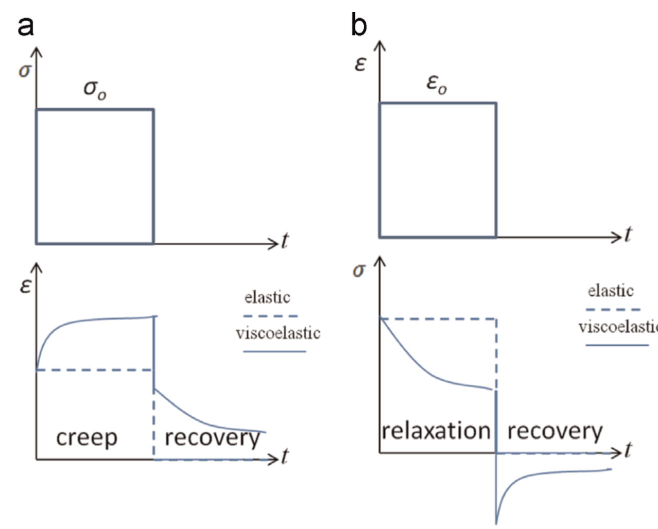

Fig. 1. Original un-deformed state of the material is shown in the top row, while (a) creep and recovery, and (b) relaxation and recovery are shown for elastic and viscoelastic material. Stress is denoted by (σ) and strain by (ε) *versus* time *t*.

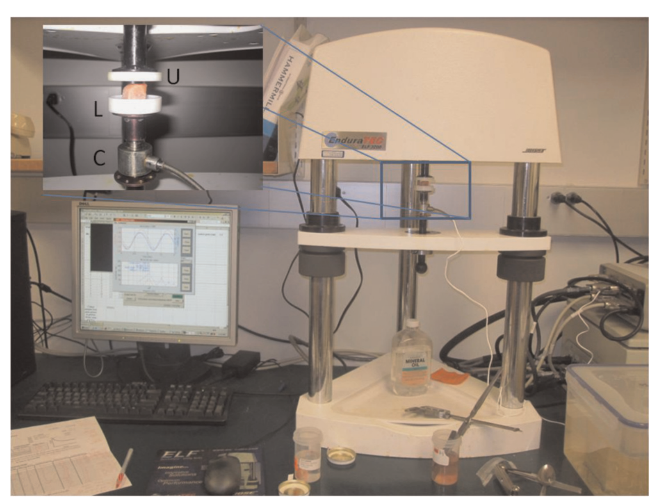

Fig. 2. EnduraTEC ELF 3200 Mechanical Testing System (MTS). The zoomed in area shows the upper platen (U), the lower platen (L), and a 1000 g load cell (C).