Multi-modal magnetic resonance elastography for noninvasive assessment of ovarian tissue rigidity in vivo

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ABSTRACT

For centuries, physicians have relied on touch to palpate tissue and detect abnormalities throughout the body. While this time-tested method has provided a simple diagnostic examination for large, superficial abnormalities, it does not permit quantifiable measurements of stiffness in deeper, small organs. Advances in noninvasive imaging to measure tissue rigidity represent important extensions of manual palpation techniques. Tissue fibrosis occurs with age in many organs; in the ovary, it is thought to be a marker of polycystic ovary syndrome and age-related idiopathic infertility, although quantitative assessment of fibrosis in this deep, abdominal tissue has not been possible. We used noninvasive methods to quantify ovarian tissue rigidity and clarify the role of tissue stiffness in reproductive health. With proper validation against accepted standards, noninvasive imaging techniques may become the quantitative counterpart to interior probing palpation methods and invasive (surgical) diagnoses, with applications across many clinical settings, including evaluation of adolescent and young adult ovarian function.

1. Introduction

The characterization of ovarian tissue rigidity is critical for understanding organ function and diseases such as polycystic ovary syndrome (PCOS) [1]. Unlike the breast or liver, however, the ovary is not typically biopsied for the diagnosis of PCOS, and clinicians must rely on external palpation to detect ovarian tissue abnormalities. Moreover, the ovary is not a regenerating tissue system, and biopsy of the ovarian tissue is thus not an option in young, normally cycling women. Noninvasive measures of ovarian function and rigidity would make it possible to more quantitatively assess mechanical properties of the ovary and diagnose diseases such as PCOS. Moreover, noninvasive measures of ovarian function in adolescents would be far superior to invasive techniques, especially those that require transvaginal probes.

Quantitative methods to measure the mechanical properties of deep, abdominal, soft tissues such as the ovary are still in their infancy [2]. Tissue size, heterogeneity, depth and porosity present challenges when attempting to accurately assess the material properties of a tissue. Tissues are complex materials and contain fluid – up to 90% or more of total weight – which greatly influences their properties and renders most tissues nearly incompressible ($\upsilon \approx 0.50$) [3]. Because of their solid–fluid nature, tissues are more accurately characterized in the frequency domain using viscoelastic material models [4]. Stiffness is measured and reported as the storage modulus (E' or G') in the frequency domain. Young's modulus (E) and shear modulus (G) are related by the Poisson ratio for isotropic homogeneous materials ($E = 2G*(1 + \upsilon)$), typically using a factor of 3 for soft materials such as tissues. Borrowing from the characterization of traditional heterogeneous materials [5–7], contact or indentation-type measurement methods have been applied to tissues [8–11] to characterize spatially resolved properties. However, this indentation-type methodology is only applicable to in vitro studies and therefore represents a quantifiable, but micro-scale, palpation test that is not amenable to clinical assessment of the ovaries.

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Magnetic resonance elastography (MRE) is a novel technique that has been used to acoustically probe internal organs, such as the breast and liver, for early detection of cancer and cirrhosis [12]. This method combines mechanical wave propagation methods with magnetic resonance imaging (MRI). During MRE, a low-frequency sound wave (60–120 Hz) is sent into the body through a driver that is placed on the body, pointing towards the area of interest. The sound wave displaces the tissue and the tissue displacements are then measured via MRI and transformed into an equivalent stiffness image, or elastogram. MRE, like MRI, is noninvasive and well accepted by patients [2]. MRE can have up to sub-millimeter to millimeter resolution given the robust algorithm package capable of reducing artifacts and directional filtering of shear waves to eliminate shear wave interference. The resolution of MRE is typically one-fifth to one-half the resolution of the MRI, image depending partially on the vibrational frequency of the acoustic wave. MRE represents a technological link between what has been accomplished previously with in vitro imaging and the ability to detect tissue abnormalities through traditional palpation. The ability to detect tissue stiffness abnormalities in vivo, which are measured as hard or soft domains on MRE, can provide important additional prognostic information and insight into the overall health of an individual patient.

Though imaging methods to date have been restricted to the assessment of the larger organs such as the liver and lungs, recent developments in driver and software technology are making it possible to penetrate deeper to analyze organs like the ovary. To date, MRE results have not been quantitatively validated by established mechanical characterization methods and have not been applied specifically to assess ovarian rigidity. Our goal was to investigate MRE as a quantitative biomaterial characterization method that could be applied clinically to assess rigidity of the ovary in vivo. We first performed a rigorous validation of MRE on gels and tissues and then compared the results to those of in vitro studies using bovine ovaries. Finally, we examined the ability of MRE to distinguish between healthy and PCOS ovaries in vivo.

2. Materials and methods

2.1. Animal tissue collection

Bovine ovaries were isolated from heifers (<2 years of age) following kosher slaughter from the Aurora Packing Company in Aurora, IL. The ovarian tissue was immediately stored at 4 °C and transported by automobile to Northwestern University (1 h).

2.2. Human tissue collection

Using an institutional review board (IRB)-approved, HIPAA-compliant protocol, seven women were selected and gave consent to undergo clinical testing with MRE (see Supplementary information). Each subject underwent multiple MRE scans to determine interscan variability.

2.3. Agar hydrogel sample processing

Granulated agar (Fisher BioReagents, Waltham, MA) was mixed with double deionized water at 2.25 wt.% for MRE, nanoindentation and bulk experiments. The gels were cured using the liquid setting in a standard autoclave for 1 h.

2.4. Bulk material characterization of gels

RSA III dynamic mechanical analysis (DMA; TA Instruments, New Castle, DE) was used to test the tensile and compressive viscoelastic properties (E', E'') of the agar hydrogel samples (phantoms). For rheometry, a series of five agar gels specimens were cut using a cylindrical die and tested for shear viscoelastic properties (G', G'') using a Physic MCR-300 (Anton Paar, Graz, Austria). Specimens were preloaded with 0.2 N to ensure proper contact before samples were subjected to testing. The details of this protocol are included the Supplementary information.

2.5. Nanoindentation

Indentation testing was performed using a Triboindenter (Hysitron, Minneapolis, MN) and a 100 μm radius spherical tip with a dynamic oscillatory load [4] of 1.0 μN at 10–200 Hz, at a sufficiently large contact depth of between 1.5 and 2 μm. For bovine ovary samples, nanoindentation tests were performed at three different sites on each ovary to test for heterogeneities: the cortex, the medulla and the corpus luteum. At each site, a series of five different sites were tested (10 data points per site), sufficiently spaced by 50–200 μm. During testing, samples were partially submerged in Dulbecco's phosphate-buffered saline to prevent dehydration. For nanoindentation testing, a universal surface energy term, similar in value to those defined for the agar hydrogels, was used to correct stiffness values to account for work of adhesion (see Supplementary information).

2.6. MRE

Measurements of internal ovarian stiffness were obtained using MRE by determining a circular region of interest within the ovary margins to ensure that the surrounding tissue was not included in the stiffness measurement. This is a conservative approach that sacrifices the outer cortex region of the ovary and only captures information on the interior region. Images were taken with a field view of 275 mm × 400 mm and were acquired on a 141 × 256 matrix, yielding anatomic images with 1.9 mm × 1.5 mm resolution, corresponding to elastograms of 1.3 cm × 1.0 cm. Stiffness was computed using the MRE software package [13] (see Supplementary information for more details on the stiffness extraction method).

2.7. Statistical analysis

Samples were compared using two-tailed Student's t-tests. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Validation of MRE in agar hydrogel phantoms

We first compared MRE, nanoindentation testing and bulk mechanical methods applied to agar hydrogels, a common tissue mimic, using a suite of testing methods covering all strain modes (tension, compression, shear) and a wide range of probing volumes. We observed considerable agreement between MRE and bulk mechanical measurements (≈15% error) (Fig. 1, left), thereby validating MRE against the standard testing methods. The minor variations in observed stiffness values can be attributed to testing differences, such as strain mode (tension/compression), sample geometries or sample variation. Nanoindentation results on the agar hydrogels, corrected using a version of the Johnson–Kendall–Roberts (JKR) model [14,15] (Fig. 1, right), match those of the bulk methodologies, especially for compression (≈8% error).