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Micro-elastography of biopsy tissue, application to endometriosis.

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1. Introduction

Endometriosis is a chronic, inflammatory and oestrogen-dependent gynaecological disease histologically characterized by the presence of endometrial-like tissue outside the uterus, which forms lesions. These lesions are ectopic endometrium as opposed to the eutopic endometrium, which physiologically lines the inner wall of the uterus and allows the embryo implantation (Taylor, Kotlyar, & Flores, 2021). Endometriosis affects 10% of women of childbearing age (Taylor, Kotlyar, & Flores, 2021). Patient quality of life may be significantly impaired mainly due to a wide range of painful symptoms (acute and chronic pelvic pain, cyclic digestive tract pain, severe period pain, painful sex and urination) and infertility (Saunders & Horne, 2021). This is a major public health problem.

Due to the inflammatory component of the disease, fibrosis is a molecular feature of the pathophysiology of endometriosis (Burney, 2022). Fibrosis, by increasing tissue stiffness, can compromise the local diffusion of drugs used to treat the symptoms of the disease and make surgical management complex (Burney, 2022). Developing methods for phenotyping endometriosis lesions from a mechanical point of view is therefore of interest, to provide a predictive view of the response to treatment.

Biopsy examination consists in collecting a small piece of tissue of interest from the patient. It is notably used in endometriosis patients for diagnostic and follow-up purposes. The addition of mechanical characterization of the endometrium from the collected tissue could enable patients to be monitored without the need for additional invasive procedures.

Shear wave elastography is one of today's leading techniques for probing the elasticity of biological tissues. It is used, for example, on the liver, to probe the liver elasticity non-invasively and diagnose

cirrhosis. Shear waves propagation is determined by the viscoelastic properties of the tissue. In particular, shear waves velocity is directly related to the Young modulus of the tissue. Measuring the velocity of shear waves means measuring the tissue elasticity.

Shear wave elastography is generally used on whole organs or centimetric samples. In fact, the wavelengths considered must be small compared to the size of the sample. In other words, the smaller the sample, the higher the shear waves frequencies considered. However, biological tissues strongly attenuate high frequencies. The challenge of micro-elastography is to observe high frequencies, close to the limit of evanescence in tissue. The combined use of a high-speed camera, a microscope and a high-frequency shear wave field has already enabled the mechanical characterization of millimeter and micrometer samples (Laloy-Borgna, Zorgani, & Catheline, 2021). To our knowledge, the smallest structures probed by dynamic microelastography are mouse ovocytes, a hundred micrometers wide (Grasland-Mongrain, et al., 2018). Biopsy samples are much larger, millimetric and therefore good candidates for characterization.

Our aim is to use dynamic micro-elastography techniques to probe the elasticity of endometrium sample harvest from biopsy examination in patients affected with endometriosis. In this work, murine endometrial samples are considered as a proof of concept. A comparison is made with murine liver tissue.

2. Methods

2.1 Samples

The mouse uterus and liver are harvested after death and stored on ice. The mouse's uterine horns are scraped with a scalpel to remove the protective membrane, leaving only the endometrium. The tissue is then cut into 1 mm³ samples. Liver samples are left for a few moments in water to remove blood and increase their transparency. 1% agarose gel at 40°C is poured into Petri dishes. Samples are placed in the gel while it is still liquid. Time between removal of tissue from ice and measurement is less than 10 minutes.

2.2 Micro-elastography platform

Figure 1A illustrates the platform. An inverted microscope (Nikon Ti-S) coupled to an ultra-fast camera (Phantom v12.1) enables us to record x10 zoom films of the sample at 20 000 frames/s. Frames are 512x512 pixels. A shaker (Cedrat APA100M) driven by a generator is placed in contact with the gel. The shaker delivers vibrations between 3 and 8 kHz. The length of acquisition is 100 ms.

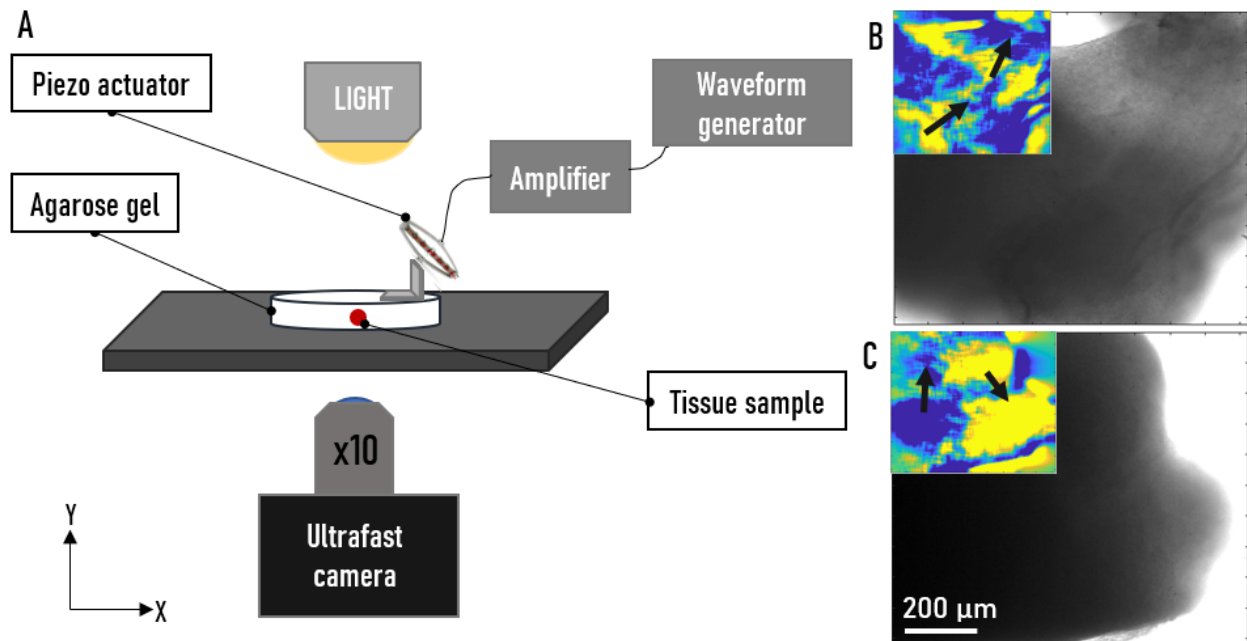


Figure 1 (A) Micro-elastography platform: a wave field created with a shaker propagates in a gel containing a murine tissue sample. Film of the propagation is acquired using an ultrafast camera. (B) Raw image obtained from an endometrial sample and an example of X-component the displacement field obtained in this sample. (C) Raw image obtained from an endometrial sample and an example of Y-component the displacement field obtained in this sample. The arrows indicate the direction of propagation. Colours are arbitrary unit.

2.3 Post processing

Figure 1B, 1C are examples of x10 magnified images obtained in an endometrium and liver sample respectively. The first step is to track the waves from the raw image stack. The displacements are computed through a phase tracking algorithm. It consists in computing the Hilbert matrix of the raw image stack to obtain the phase in each point. Then the phase difference is computed frame by frame.

Displacements are computed in both X and Y direction. Then the displacements are filtered on a band of interest (3-8 kHz). The signal is then whitened on the band of interest. Examples of the displacements field obtain in endometrium and liver samples are given in Figure 1B, 1C.

2.4 Shear wave velocity

To obtain the propagation speed of shear waves, we use so-called "noise correlation" algorithms, developed in the seismology field. We assume a diffuse wave field, containing all possible directions of propagation. These algorithms allow the wavelength to be traced back through a description of the wave field as the time-turned field (Catheline, et al., 2013). The temporal cross correlation of one point in space with all the others points is approximated as the point spread function of the medium. The wavelength is approximated as the half-height width of the point spread function. The wavelengths found at each point in space are averaged to obtain a mean value for the

sample. The waves center frequency is estimated as the center frequency of the bandwidth over which the signal has been whitened. Shear wave velocity is then calculated by multiplying wavelength and frequency.

3. Results and discussion

The endometrium of 3 different mice was studied. For each individual, 3 samples were taken and an acquisition was performed on each sample. Similarly, the liver of 3 different mice was sampled. For each individual, 3 samples were taken and an acquisition was performed on each sample.

On the basis of preliminary data acquired, the liver appears significantly harder than the endometrium. Quantitative estimates of elasticity are forthcoming.

4. Conclusions

A method for the mechanical characterization of biopsy samples is proposed. Preliminary results obtained in murine liver and endometrial samples are very encouraging. There appears to be no barrier to the mechanical characterization of endometrium sample harvested from patient affected with endometriosis. What's more, this method can be used for all types of biological tissue, opening the way to rapid mechanical characterization of biopsies.

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Conflict of Interest Statement

The authors have no conflicts to disclose

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