Estimating localized oscillatory tissue motion for assessment of the underlying mechanical modulus

E.E. Konofagou a,b,*, M. Ottensmeyer c, S. Agabian b, S.L. Dawson c, K. Hynynen b

a Department of Biomedical Engineering, Columbia University, New York, NY 10027, USA
b Focused Ultrasound Laboratory, Department of Radiology—MRI research, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA 02115, USA
c Simulation Group, CIMIT, Cambridge, MA 02139, USA

Abstract

The technique of harmonic motion imaging (HMI) uses the localized stimulus of the oscillatory ultrasonic radiation force as produced by two overlapping beams of distinct frequencies, and estimates the resulting harmonic displacement in the tissue in order to assess its underlying mechanical properties. In this paper, we studied the relationship between measured displacement and stiffness in gels and tissues in vitro. Two focused ultrasound transducers with a 100 mm focal length were used at frequencies of 3.7500 MHz and either 3.7502 (or 3.7508 MHz), respectively, in order to produce an oscillatory motion at 200 Hz in the gel or tissue. A 1.1 MHz diagnostic transducer (Imasonics, Inc.) was also focused at 100 mm and acquired 5 ms RF signals (pulse repetition frequency (PRF) = 3.5 kHz) at 100 MHz sampling frequency during radiation force application. First, three 50·50 mm² acrylamide gels were prepared at concentrations of 4%, 8% and 16%. The resulting displacement was estimated using crosscorrelation techniques between successively acquired RF signals with a 2 mm window and 80% window overlap at 1260 W/cm². A normal 1-D indentation instrument (TeMPeST) applied oscillatory loads at 0.1–200 Hz with a 5 mm-diameter flat indenter. Then, 12 displacement measurements in 6 porcine muscle specimens (two measurements/case, as above) were made in vitro, before and after ablation which was performed for 10 s at 1260 W/cm². In all gel cases, the harmonic displacement was found to linearly increase with intensity and exponentially decrease with gel concentration. The TeMPeST measurements showed that the elastic moduli for the 4%, 8% and 16% gels equaled 3.93±0.06, 17.1±0.2 and 75±2 kPa, respectively, demonstrating that the HMI displacement estimate depends directly on the gel stiffness. Finally, in the tissues samples, the mean displacement amplitudes showed a twofold decrease between non-ablated and ablated tissue, demonstrating a correspondence between the HMI response and an increase in stiffness measured with the TeMPeST instrument.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Displacement; Modulus; Oscillatory; Radiation force; Soft tissue

1. Introduction

Palpation as a diagnostic tool makes use of the differences in mechanical properties between tumors and their surrounding tissues. This is especially true in the breast. Infiltrating ductal carcinomas have been found to have average moduli of 558 ± 180 kPa compared with 48 ± 15 and 20 ± 8 kPa for normal glandular and fat tissue in the breast [1]. As a result, several methods have been developed to estimate tissue stiffness, or stiffness-dependent tissue responses, following a mechanical stimulus.

One method that induces vibration remotely and detects tissue mechanical properties is ultrasound-stimulated acoustic emission [2]. This method uses ultrasound induced radiation force to probe tissue properties. As an ultrasound beam propagates through the tissue, part of its energy is absorbed and part of it scattered away. The momentum change of the beam results in a force that acts on the tissue. In this technique, two ultrasound beams operating at slightly different frequencies \( f_1 \) and \( f_2 \), overlap and interfere at the focal region generating a wave that is amplitude modulated at their difference frequency \( f_d = f_2 - f_1 \). An object at the overlapping zone experiences an average energy density of

0041-624X/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
that fluctuates at \( f_d \). This varying force causes the tissue to oscillate at the frequency \( f_d \) and thus, acts as an acoustic source. The magnitude of the acoustic source depends on the radiation force and the mechanical sinusoidal response of the tissue at the frequency \( f_d \). The stimulated acoustic signal propagates through the tissue and can be detected by an external hydrophone [2]. In fact, the technique has, for example, been proven successful in the detection of microcalcifications in breast tissue. The resulting acoustic signal is affected by the mechanical and acoustical properties of the oscillating (tumor) tissue, the water tank resonance (in which these tests are performed), the characteristics of the surrounding tissue and the signal's interaction at the hydrophone. Therefore, stiffness estimation using this method is extremely challenging. This is because the absorption and stiffness of tumor tissue, which are different from normal tissue or microcalcifications, can have opposite effects on the acquired USAE signal [3]. Thus, there is still a need for a technique that locally applies the force, but only measures a response that is solely dependent on the stiffness parameter for tumor detection.

In this study, in order to avoid the artifacts and drawbacks of the overall USAE application, we propose to use the mechanical excitation generated by that technique but not its method of measurement of the tissue response (Fig. 1). All the advantages of the USAE technique without the ambient noise drawbacks are thus maintained. The development of localized harmonic motion imaging (HMI) has already been described [4]. The main principle behind the proposed technique is that local harmonic motion induced by an oscillatory, remotely applied, harmonically varying radiation force of amplitude \( F_0 \), can be precisely estimated and imaged in tissues (Fig. 2). Until now, techniques have either involved the estimation of motion using an external vibration or have estimated localized static motion. In this study, we use the radiation force applied by overlapping two beams radiating at slightly different frequencies, same as the method in [2]. However, the harmonic motion is estimated at different snapshots of the motion \( (t_1, t_2, \text{etc.}) \) using crosscorrelation of RF ultrasonic signals acquired by a separate diagnostic ultrasound beam focused at the location undergoing vibration (Fig. 3). This method is distinctly different from methods such as remote palpation [5] and shear wave elasticity imaging (SWEI) [6] that estimate motion after removal of the force instead of during its application, such as is the case of USAE and HMI. Therefore, the response is therefore more dependent on the lesion or tumor than on the perilesional tissue. In addition, the local elastic modulus \( E \) can be directly estimated from the characteristics of the harmonic motion [1], i.e.,

\[
E = \frac{2(1 - v^2)F_0r}{X_0A},
\]

where

\[
F_0 = \frac{2\pi I}{c}
\]

Fig. 2. Localization of the radiation force in the tissue (upper block) produced by the FUS transducer(s) at the lower (water) block of the image. Image courtesy of Chris Connor [9].

Fig. 3. Concept of Harmonic Motion Imaging for displacement estimation. RF line tracking at different instants \( (t_1, t_2, \text{etc.}) \) acquired at the focus of the diagnostic transducer (Fig. 1) yields precise displacement estimates and identifies the characteristics of the locally induced vibration.

Fig. 1. Harmonic motion imaging setup.
with $A$ is the cross-sectional area of the beam at the focus, $r$ is the radius of the beam at the focus, $x$ is the absorption of the tissue, $I$ is the intensity of the radiation force-generating transducer(s) and $c$ is the speed of sound [5]. In this paper, the dependence of the HMI response on the underlying elastic modulus is examined using gel phantom and in vitro tissue experiments.

2. Methods

2.1. Gel experiments

Three $50 \times 50$ mm$^2$ acrylamide gels were prepared at concentrations of 4%, 8% and 16% following Protocol 2 in [10] and using the proportions as shown in Table 1. The resulting displacement was estimated using cross-correlation techniques between successively acquired RF signals with a 2 mm window and 80% window overlap at 1260 W/cm$^2$. Sephadex (Sigma Aldrich, Inc.) particles were added in the gel phantoms for scattering [4]. Two focused ultrasound transducers were used, the first operating at a frequency of 3.75 MHz and the second at 3.7502 (Fig. 1). A PZT composite diagnostic transducer (Imasonics, Inc.) was operated in pulse/receive mode at a frequency of 1.1 MHz and focused at a depth of 10 cm. The focused transducers and the diagnostic probe were all focused on the same region of the tissue-mimicking gel phantoms in order to maximize the signal-to-noise ratio of the measured gel motion (Fig. 3). The pulse duration was equal to 0.28 ms and a PRF of 3 kHz was used. RF data was acquired at a sampling frequency of 50 MHz for a duration of 10 ms, digitized on a digital oscilloscope (Yokogawa DL 7100, Tokyo, Japan) and stored on disk. RF signal tracking was performed using cross-correlation techniques with a window on the order of 1–2 mm. Estimates of the displacement relative to the initial position (i.e., at the onset of the application of the radiation force) were obtained during the application of the radiation force that oscillated at 200 Hz in order to maintain consistency with the mechanical testing parameters. The focused ultrasound intensity used was approximately equal to 1260 W/cm$^2$. Prior to displacement estimation, a notch filter was used to remove the fundamental frequency of the radiation force generating transducers (in this case 3.75 MHz) and all its harmonics from the signals. The displacements were also imaged in an M-mode-like fashion in order to study both their spatial and temporal variation [4].

As an independent technique for measuring the tissue elastic modulus, an indentation instrument, the TeM-PeST 1-D (Fig. 4 [7]), was employed to determine the tissue sample impedance from 0.1 to 200 Hz. This instrument applies a load to the tissue sample surface with a 5 mm right circular indenter, while recording the applied force and relative motion away from an initial indentation caused by an applied preload force. For special geometries and approximations regarding the behavior of the tissue, a number of closed form solutions relate impedance (compliance) to the underlying material properties. If a sample is approximately semi-infinite (i.e. the deformation and indenter are small relative to the sample), linearly elastic, homogeneous and

Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gel concentration (in 96 ml water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4%</td>
</tr>
<tr>
<td>Acrylamide (g)</td>
<td>3.8</td>
</tr>
<tr>
<td>Bisacrylamide (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>TEMED in 12 ml water (ml)</td>
<td>0.12</td>
</tr>
<tr>
<td>SPS in 6 ml water (g)</td>
<td>0.12</td>
</tr>
<tr>
<td>De-ionized Water (ml)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Fig. 4. (a) TemPesT setup; (b) Gel and indenter detail.
isotropic, and the contact is frictionless, then elasticity and compliance are related as: $E = (1 - v^2)/dc$, where $E$ is Young’s Modulus, $v$ is the Poisson’s ratio, $d$ is the indenter diameter and $c$ is the measured compliance (inverse of spring constant). Many tissues are found to be approximately incompressible, so $v$ is often assumed to have a value close to 0.5.

In these experiments, it is recognized that tissues are typically not linear and that they are visco-elastic, so this expression is used to convert frequency-dependent compliance to an expression for apparent elasticity, which may differ from true elasticity, but is useful as a first approximation. Additional accuracy can be obtained by employing finite-element techniques, using the measured stiffness and geometry as inputs, and the material parameters as outputs (the “inverse” problem); this additional analysis will be warranted when further data have been collected. To capture the visco-elastic and inertial character of the tissue, a modified Voigt model was used [8], which included a lumped mass element to represent the density and effective volume of the tissue deformed/vibrated by the indenter. It was assumed that for a given preload the stiffness was locally linear (i.e. by linearizing about a specific point on a non-linear force/displacement characteristic curve), which permitted calculation of the effective mass from the limiting value of the static compliance and the natural frequency of the response. The damping coefficient can be determined from the ratio between the static and peak values of the compliance [8]. From these relations, an expression for material elasticity can be determined as a function of frequency and applied mean stress.

2.2. Tissue experiments

Samples of porcine muscle were obtained from recently euthanized subjects of unrelated experiments and immediately immersed in 0.9% saline. Within an hour post mortem, these samples were prepared by cutting them into slabs with approximately parallel upper and lower surfaces. They were placed on rigid substrates, and tested with the TeMPeST instrument using a “chirp” signal—a sinusoidal force with a monotonically increasing instantaneous frequency. Nominal input preloads were 30, 60 and 120 mN, with force amplitudes of 30 mN. This would ensure continuous contact between the indenter tip and the tissue surface. The frequency range of the chirp was 0.1–200 Hz, and chirps of both increasing and decreasing frequency were employed to avoid any effect of tissue creep, which might be observed as a decrease in measured stiffness from the beginning to the end of a chirp—a change related to time, but not the instantaneous frequency.

Subsequent to these initial tests, HMI measurements were made using the same parameters as those used in the gel experiments and at the same location of the muscle sample as that of the TeMPeST measurement. A lesion was then created at the same location (on the surface of the tissue) using an ultrasonic intensity of 1260 W/cm² for 20 s. Ultrasonic signals were acquired and HMI displacements were then estimated using the same parameters as those used before ablation. The stiffness was evaluated using HMI, after which the indentation tests were repeated directly over the lesion location. The length scale of the lesion was approximately the same as the diameter of the indenter tip (5 mm in diameter). Finally, 12 HMI displacement measurements in six in vitro porcine muscle specimens (two measurements/specimen) were made before and after ablation without any mechanical testing verification. All remaining parameters were identical to those used in the gel experiments.

3. Results

3.1. Gel experiments

Fig. 5 shows the compliance variation with frequency in all three plots. Following the model discussed in the methods section, the TeMPeST measurements showed that the elastic moduli for the 4%, 8% and 16% gels (Table 1) equaled 3.93 ± 0.06, 17.1 ± 0.2 and 75 ± 2 kPa, respectively. The variation of the HMI displacement amplitude with the measured gel modulus are shown in Fig. 6. A steady decrease in amplitude was noted with increasing stiffness, similar to that observed in the case of the simulations [4].

3.2. Tissue experiments

Fig. 7 clearly shows that compliance falls (stiffness increases) with increasing preload. This corresponds with expected non-linear stiffness in the tissue and due to geometric changes (i.e. deeper indentation). The natural frequency (and effective mass/volume) also change as geometry and vibration amplitude change. The lumped parameters obtained for the various cases are shown in Table 2, as are the estimated values of the modulus of elasticity. Interestingly, the tissue under higher preloads exhibits behavior which has features similar to a Kelvin (standard linear) tissue model augmented with an iner-
This model includes an additional damping term, and fitting the data to this model will be performed as additional data becomes available. Fig. 8 shows the variation of the HMI displacement amplitude between non- and ablated tissue. The mean displacement amplitude showed a twofold decrease between non-ablated and ablated tissue, thus depicting the stiffness dependence of the HMI response in tissues. The low modulus variation between normal and ablated tissue as measured by TeMPeST may be due to the fact that the ablated tissue is much less homogeneous and therefore the model assumptions as described in the methods section no longer hold. However, HMI measurements are more localized showing a higher percent decrease. Investigations are currently ongoing in order to investigate this effect.

4. Discussion

Harmonic Motion Imaging is a new technique that applies an oscillatory radiation force at large depths in tissues using two focused ultrasound transducers and estimates the resulting motion using a diagnostic ultrasonic transducer. It has previously been shown using simulations and preliminary experiments that the estimated HMI amplitude decreases with increasing tissue modulus. In this paper, this observation is verified experimentally using independent mechanical measurements. A normal indentation system (TeMPeST 1-D) was employed to measure the modulus of gels at different acrylamide concentrations, and porcine muscle tissue in vitro before and after coagulation. In both cases, the HMI displacement amplitude was shown to decrease with the measured modulus; reinforcing the premise of HMI for direct tissue modulus estimation. Future studies will include modification of the model.
used to estimate the modulus with TeMPeST in order to account for the inhomogeneity in tissues resulting from ablation. This should also provide mechanical measurements that are better correlated with the HMI localized estimates.

Acknowledgements

This study was supported by grants from the Brigham Research and Education Fund, the Radiological Society of North America and NIH R21 CA82275.

References