MR Elastography and Diffusion-Weighted Imaging of ex-vivo Prostate Cancer: quantitative comparison to histopathology

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Abstract

Purpose—1) to develop a Magnetic Resonance Elastography (MRE) system for imaging of the *ex-vivo* human prostate, 2) to assess the diagnostic power of mono-frequency and multi-frequency MRE and diffusion weighted imaging (DWI) alone and combined as correlated with histopathology in a patient study.

Materials and Methods—An electromagnetic driver was designed specifically for MRE studies in small-bore MR scanners. *Ex-vivo* prostate specimens (post-fixation) of fourteen patients who underwent radical prostatectomy were imaged with MRE at 7 T (nine cases had DWI). In six patients, the MRE examination was performed at three frequencies (600, 800, 1000 Hz) to extract the power-law exponent Gamma. The images were registered to wholemount pathology slides marked with the Gleason score. The areas under the Receiver-Operator-Characteristic curves (AUC) were calculated.

Results—The methods were validated in a phantom study and demonstrated that (i) the driver does not interfere with the acquisition process, (ii) the driver can generate amplitudes greater than 100 μm for frequencies <1kHz. In the quantitative study, cancerous tissue with Gleason score at least 3+3 was distinguished from normal tissue in the peripheral zone with an average AUC of 0.75 (G_d), 0.75 (G_l), 0.70 (Gamma- G_d), 0.68 (ADC), and 0.82 (G_d+G_l+ADC). The differentiation between PZ and CG was modest for G_d ($p<0.07$), G_l ($p<0.06$) but not significant for Gamma ($p<0.2$). A correlation of 0.4 kPa/h was found between fixation time of the prostate specimen to the stiffness of the tissue which could affect the diagnostic power results.

Conclusion—DWI and MRE may provide complementary information; in fact MRE performed better than ADC in distinguishing normal from cancerous tissue in some cases. Multi-frequency (Gamma) analysis did not appear to improve the results. However, in light of effect of tissue fixation, the clinical implication of our results may be inconclusive and more experiments are needed.

Keywords

Prostate Imaging; MR Elastography; *ex-vivo* Prostate Cancer

INTRODUCTION

Magnetic Resonance Elastography (MRE) is a method to probe the mechanical properties of tissue and has been shown to be capable of diagnosing diseased tissue (1,2). In MRE, an external mechanical excitation is applied to the tissue of interest to induce vibrations. The resulting wave fields are imaged using a motion-sensitized MRI pulse sequence synchronized with the mechanical excitation. The mechanical properties of the tissue of interest are calculated from the wave fields and displayed as an image commonly referred to as an elastogram. It is hypothesized that information of mechanical properties extracted using MRE may further increase the sensitivity and specificity of detecting and staging prostate cancer in multi-parametric MRI. In this work, we report the development of a transducer to apply tissue excitation in a 7T MRE system. Using this system, dynamic MRE was performed along with DWI on *ex vivo* prostate specimens in an effort to determine if the methods present complementary information which may be used to improve diagnostic power in *in-vivo* multi-parametric imaging. To compare the results of the imaging with histopathology, imaging and pathology were carefully registered by (i) aligning the MRI scan with the specimen and using a special whole-mount sectioning device (3) and (ii) using a volumetric contour-based alignment between the MRI scan and the whole mount histopathology slices (4).

Previously, Dresner *et al.* (5) applied dynamic MR elastography to seventeen post-mortem *ex-vivo* human prostate cancer specimens at 1.5 T at several excitation frequencies (100–600 Hz). This study did not compare the elastograms to histopathology. In another study by Dresner *et al.* (6), seventeen fresh specimens resected for adenocarcinoma were assessed with MRE and compared to histology slides that were manually matched with corresponding anatomy images. Although this study compared the results to histopathology, image registration and a voxel by voxel approach was not used. Most recently, McGrath *et al.* (7,8) used quasi-static MRE (1–5 Hz) methods at 7T to study effects of pathology before and after fixation on *ex-vivo* human and canine prostate specimens. Again, the resulting strain/elasticity maps were not compared to histopathology.

In this article, first the setup and driver mechanism of a transducer suitable for small bore 7T MRE is discussed. The imaging approach was validated in a phantom study. Then the results of the *ex-vivo* study are presented and discussed. A comparison of our results with other published work, as well as the limitations of this study are presented in the discussion section. This work represents the first study comparing mono- and multi- frequency MRE results (alone and in combination with DWI) to whole-mount histopathology on a voxel-by-voxel basis.

Methods

Patient study

Ethics board approval and informed consent were obtained from fourteen patients scheduled for radical prostatectomy recruited between December 2011 and February 2013. Following surgery, the prostate specimens were immersed in formalin for various times as listed in Table 1. The MRI experiments were performed after the fixation process. In two cases,

incomplete data was acquired due to technical issues and were not included in the study. In one patient (P13) the Gleason score was not assigned by the pathologist due to previous androgen therapy, and thus was not included in the analysis. The analysis in this article is performed on the remaining twelve patients (mean age of 66 yrs). Clinical data including fixation time, gland size, prostatectomy Gleason scores, and clinical stage, the number and size of tumors, and notes from Pathology for the sixteen patients who had biopsy confirmed cancer are summarized in Table 1.

Mechanical transcoder and experimental setup

Figure 1 shows the electromagnetic transducer designed specifically for a small-bore MRI scanner. The driver utilizes the magnetic field of the scanner itself to drive a swiveling current carrying coil. The driver is placed outside the scanner at the fringe field as shown in Figure 1a. The excitation is applied to the specimen via an adjustable mechanical extension (8mm diameter rigid carbon fiber tube). The pre-compression on the specimen can be adjusted, and in this case we applied approximately 5N of constant force on the tissue. Pre-compression is achieved by rotating a thumbscrew that presses the carbon fiber tube to the specimen via a flexural spring. The driver does not contain any ferromagnetic or permanent magnetic material that could pose a potential risk to researchers setting up the driver (9). A common issue with electromagnetic drivers is the unwanted interaction of the switching gradient field with the excitation coil (10–12). This results in spurious mechanical excitation when the excitation coil is placed inside the bore of the scanner. Here, we avoided this issue by placing the mechanical driver outside the bore and at the fringe field of the scanner. In our recent publication (13) we showed that having a separate stationary coil wound concentric to the vibrating coil can help eliminate unwanted magnetic field generated by the moving coil when it is within 40cm of the specimen. The relative size and number of turns of the coils were optimized using finite element modeling. The amount of current needed for sufficient wave amplitude in real tissue (especially stiff tissue) is much larger compared to, for example, a gelatin phantom. In addition, excitations of a few kilohertz also require larger currents to pass through the coils which would again increase the unwanted magnetic fields. The actuator as presently designed is shielded and therefore large currents can pass through the coils to provide significant forces at high frequencies without unwanted magnetic fields.

The spool is made of a Granolite[®] G10 Material, which is known for its mechanical stability even at elevated temperatures. The U-joints are made of Acetal plastic, and the rest of the driver parts are made of water jet-cut polycarbonate sheets held together by nylon fasteners. As a safety feature, two thermal components are incorporated into one of the coils to protect the driver in the event of overheating. A thermal cut-off switch opens the circuit if the temperature exceeds 60° C. This switch is slow acting and resets the circuit if the temperature drops below the cut-off. The second sensor is a fast-acting thermal fuse that permanently disconnects the circuit if the temperature reaches 150° C.

The electronics consists of an arbitrary waveform generator (Agilent 33220A, USA), which is triggered by the MR scanner; a 2×440W (8Ω) switching audio amplifier (Behringer NU3000, Germany); a passive low-pass filter with a cut-off at 2.8 kHz, designed to remove switching noise from the amplifier output; and a handheld oscilloscope (Agilent U1602B,

USA), used to monitor signal levels and fine-tune the synchronisation between the MR scanner and the driving frequency. The current fuse (5 A) is incorporated into the filter module.

The *ex-vivo* prostate specimen is placed in a sealed plastic bag with a 2 mm diameter plastic rod inserted in the urethra as shown in Figure 1c and d. The anterior of the specimen is placed flush against a plastic cradle with the vibration applied to the apex. The specimen is taped to the cradle to ensure that it remains in place when the mechanical excitation is applied. The cradle is then inserted into the RF coil and secured by tape. Once the RF coil is centered in the iso-center of the scanner, the transducer rod is introduced into the cradle, fastened to the transducer, and the pre-compression is adjusted to ensure proper mechanical coupling.

Acquisition

MRI—All MRI experiments were carried out with a 7-Tesla small-bore scanner (Bruker BioSpin GmbH., Ettlingen, Germany). A standard quadrature RF coil with inner diameter of 60 mm was used. The gradient coils can generate gradient fields of up to 400 mT/m with 0.1 ms rise time. A T2-weighted Rapid Acquisition with Refocused Echos (RARE) sequence with an echo time of 70 ms, repetition time of 5 s, and RARE factor 8 was used. The total imaging time was 2.5 hours.

MRE—For MRE imaging, a conventional motion-sensitized spin-echo pulse sequence was used with $G_{\max} = 200$ mT/m (14). For the first five cases, the 3D wave field was acquired on 9 slices for a 64×64 matrix with 1 mm^3 isotropic voxels. The mechanical displacement was sampled at 8 time points per period. The heat buildup in the gradient coils limited our initial efforts to image the entire gland. For the cases performed after a hardware upgrade (gradient coils and chiller), the 3D wave field was acquired on more than 20 slices (entire prostate gland) for a 64×64 matrix with 1 mm in-plane resolution and 2 mm slice thickness. The mechanical displacement was sampled at 4 time points per period. Echo times from 35 ms to 50 ms and repetition times between 0.8–1.2 s were used. For mono frequency experiments, the mechanical excitation was 800 Hz. In six cases (P06, P08, P09, P18, P19, P20) multi-frequency experiments were performed at three acquisition frequencies of 600, 800 and 1000 Hz. The imaging time was 22 min per frequency.

DWI—for the diffusion-weighted imaging, the acquisition field of view was matched to the MRE experiments but the in-plane resolution was doubled to 0.5 mm. Four b-values of 250, 500, 750 and 1000 s/mm^2 with echo time of 21 ms and repetition time of 3.9 s were used. The apparent-diffusion coefficient (ADC) map was calculated based on a mono-exponential fit to the magnitude images of the diffusion-weighted sequence on a per-pixel basis. The imaging time was approximately 21 min. The DWI study was not performed for cases P01, P04 and P10 due to time limitations.

Image processing

A voxel was marked invalid if (i) its magnitude in the image was below a threshold of 10% of the average magnitude; or (ii) the total wave amplitude U_{tot} was less than $0.5 \text{ } \mu\text{m}$.

The viscoelastic mechanical shear properties (dynamic shear modulus G_d and loss modulus G_l) were determined using the curl-based direct inversion of the 3D wave equation assuming an isotropic linear material, that is incompressible and homogeneous (15,16). In this method, the compressional component of the wave equation is removed by applying the curl operator, resulting in a 3D shear wave equation with the complex shear modulus G as the only unknown once the tissue density is assumed. For such assumptions the wave equation becomes

$$-\rho\omega^2\vec{u} = \mu\nabla^2\vec{u} + (\lambda + \mu)\nabla\nabla \cdot \vec{u} \quad (1)$$

where ρ is density, u is the displacement, λ is the dilatational modulus and μ is the shear modulus (Lamé parameters). The second term on the right hand side can be removed by applying the curl operator to both sides of the equation which in effect removes the undesired compressional component in the measured displacements.

$$\vec{q} = \vec{\nabla} \times \vec{u} \quad (2)$$

Thus the equation to solve becomes:

$$-\rho\omega^2\vec{q} = G\nabla^2\vec{q} = (G_d + jG_l)\nabla^2\vec{q} \quad (3)$$

This results in three values for G (one for each spatial direction), that in theory are identical. In practice, however, inversion of each spatial component in of the 3D wave equation typically leads to different estimates for the viscoelastic parameters due to presence of noise. This issue is addressed by applying a constrained polynomial fit (16) to the raw data for further processing thereby reducing the effect of noise in biasing the reconstructed data.

The displacements necessary to find G from the shear-wave equation were encoded in the phase of the MRI images (a spin-echo pulse sequence with motion encoding gradients synchronized with the mechanical vibration). Prior to inversion, displacements were unwrapped (17) and then aligned in time and space to ensure no phase jumps were present. Then, the displacements were filtered by a $3 \times 3 \times 3$ spatial Gaussian filter with a 0.5 mm standard deviation. Subsequently, a constrained polynomial fit (16) was applied to the displacement field with a constraint that enforced incompressibility and isotropy condition. The fitting allows for better estimates of the first, second and third order spatial derivatives. The constraint ensures that all three spatial components of the wave equation provide the same estimate for the complex shear modulus. The main component of the displacement is extracted using a Fourier transform of the unwrapped-corrected displacement data in the time-direction. Once the spatial derivatives of the displacements are determined, the curl is calculated from equation (2) and used in equation (3) for reconstruction. Finally, the curl

operation and the inversion, the reconstructed parameters were filtered using a Gaussian filter of $3 \times 3 \times 3$ with a 1 mm standard deviation.

The dependence of each parameter on the frequency ω was modeled by a power law ($G \propto \omega^{\text{Gamma}}$), where we estimated the exponent parameter Gamma. γ - G refers to the power law exponent for G_d , and Gamma- G_l refers to the power law exponent of G_l .

Histology, registration and data analysis

Histology—Following surgery, the prostate gland was immersed in formalin solution until the day of the MRI imaging study (70 hrs on average). The fixation time ranged between 24 and 96 hours. In cases where the gland was large, the formalin was injected into the gland using a syringe. The transverse MR images were planned such that they were perpendicular to the urethra (apparent with the inserted plastic rod) and also perpendicular to the floor of the cradle. This ensured that the histology cuts are parallel to the acquired images. All subsequent images have the same orientation as this T2-weighted image. After imaging, the specimens were sliced at 4 mm intervals similar to methods discussed in (3) using a custom cutting device, in order to align the histology slides with the transverse images. The slices were then sent for whole mount histology, where an experienced pathologist marked the outline of the tumours, along with the Gleason score, on the histology slides.

Registration—The outline of the prostate was segmented on the histology slides and the T2-weighted images. An experienced radiologist verified the segmentations. The 2D segmented contours of the histopathology were then registered to the surface of prostate that was extracted from the segmented T2-weighted volume (4). The segmentation method (4) uses particle filtering to minimize the Euclidean distance between the contours and the surface with respect to in-plane translation and rotation of each histology slice alone, out-of-plane translation and rotation of all the slices together, and scaling.

ROC analysis—The analysis was performed using MATLAB® (Mathworks Inc., USA) software. For each case, the tumors marked with the Gleason score were outlined in Stradwin (Medical Imaging Group, Cambridge University, UK) to generate binary masks. The prostate capsule (PC), peripheral zone (PZ), central gland (CG) and transition zone (TZ) were identified in the T2-weighted images by an experienced radiologist for zonal analysis. After the registration process, the calculated transformation was applied to the images (T2-weighted, G_d , G_l , ADC) as well as masks to transform them to the histology slide coordinates. For tumors smaller than 4 mm in diameter the mask was dilated by approximately 1.5 mm, and for tumors larger than 8 mm the mask was eroded by approximately 1.5 mm. This was done to account for errors such as registration misalignments, and enable analysis of smaller tumors. Depending on the zonal masks (PC, PZ and CG), the mask for normal tissue was calculated by removing all the tumors marked in the histology slides and eroding the masks by approximately 3.5 mm.

The analysis for each case was performed by dividing the data in windows (size between 5–6 mm) with 10% overlap between subsequent windows, following the approach outlined in (18). A window was labeled as cancer if more than half of the window intersected with a Gleason 3+3=6 tumor; otherwise it was marked as normal. Similarly, for the zones, if more

than half of the window intersected with, for example PZ, the zone was labeled as PZ. The data was then used to calculate the receiver-operating characteristic curve (ROC), and the sensitivity, specificity and area-under-curve (AUC) values were calculated. The total number of windows used for the computation of the AUC depends on the size of the images of the scanned histology slides. In our slides we had between 60 and 150 windows per slide, depending upon the size of the slides. For example, for a 100x100 pixel image with windows that have 10% overlap, we would have $11^2=121$ windows. A Naive Bayesian statistical classifier was used to construct a model for combined G_d , G_l and ADC. The prediction from this model was used to plot the ROC and calculate the performance parameters. A Wilcoxon rank sum test was performed to test the differentiation between normal and cancerous tissue.

Phantom validation

As described in previous chapters, the methods were validated in quantitative quality-assurance phantoms (Model 049, CIRS Inc, USA) with spherical inclusions. In this study a new phantom imaging study was constructed to ensure that the inversion method can reconstruct spatially-heterogeneous inclusions which are common in real tissue. The phantom used in this experiment is an Agar-gelatin phantom with the concentration of 1% for the background and 2% for the hard inclusions. To generate T2-weighted and visual contrast, acrylic color was added to the 2% agar solution before being injected into the background solution. The inclusions were injected by a syringe before the gelatin solution was solidified. Many inclusions of various sizes and shapes were created in this manner. For the phantom MRE study, a sinusoidal mechanical excitation of 350 Hz was applied.

Results

Transducer and phantom study

The T2-weighted image, displacement field in three directions U_x , U_y and U_z , as well as the real part of the shear modulus G_d of the phantom experiment are shown in Figure 2. (with axial and coronal view). The waves uniformly distributed across the phantom and the acquired images were free of artifacts due to the electromagnetic transducer.

The harmonic distortion is the ratio of the sum of the powers of all harmonic components to the power of the fundamental frequency, and is given by:

$$THD = \frac{\sum_{k=2}^{\infty} U_k}{U_1} \quad (4)$$

where U is the wave amplitude at harmonic frequency k . This is a measured of non-linearity of the system (transducer, tissue, etc). The total harmonic distortion in the displacement field was less than 10%.

Even though not all inclusions are visible on the elastograms, there is some correspondence between the elasticity image and T2-weighted image. The output amplitude of the transducer

was $<200\ \mu\text{m}$ peak-to-peak for a frequency range between 20 Hz and 2 kHz. The transducer did not overheat even during extended examinations (2–3 hours).

Patient study

In Figure 3, a typical *ex-vivo* prostate specimen is shown with its corresponding wave images at the three excitation frequencies of 600, 800 and 1000 Hz. The figure shows the T2-weighted image, as well as the corresponding magnitude image from the MRE experiment, waves in all three dimensions, the reconstructed elastogram and the map of Gamma. The displacement field revealed that the wave propagation is complex and the waves are present in all spatial components. Also, the elasticity increased with frequency according to the power law with a mean exponent Gamma - G_d of 1.1 ± 1.3 for this case across the gland.

Sample results as registered to the pathology slides are shown in Figure 4 (MRE at 800Hz). The following images are shown in each case: the histology slides with marked tumors, the registered T2-weighted anatomy, the registered masks of the zones in the prostate, registered G_d and G_l , and ADC (where available). In terms of registration performance, the area overlap between the segmented histology and registered segmented MR slices was $94.8 \pm 3.5\%$, and the error between landmarks on histology and the corresponding registered MR was $1.8 \pm 1.1\ \text{mm}$. As seen in the figure, many the tumors can be visualized in the elasticity images even though in some cases they are not obvious in the ADC maps.

In Table 2, the average value of the dynamic shear modulus (G_d), loss modulus (G_l) and apparent diffusion coefficient (ADC) are recorded for normal and cancerous tissue in PZ and CG. In the cases where the table is empty, no tumor was found in the corresponding zone. The differentiation between normal and cancerous tissue (p-value) is also recorded in this table. We analyzed 112 tumors with an equivalent diameter between 3 and 30 mm (mean of 6 mm) and Gleason score of at least $3+3=6$. The average value of shear modulus for normal (and cancer) tissue in PZ was $65 \pm 25\ \text{kPa}$ ($69 \pm 21\ \text{kPa}$) for G_d and $56 \pm 21\ \text{kPa}$ ($58 \pm 17\ \text{kPa}$) for G_l . For CG the average shear modulus was $55 \pm 22\ \text{kPa}$ ($63 \pm 22\ \text{kPa}$) for G_d and $46 \pm 20\ \text{kPa}$ ($54 \pm 13\ \text{kPa}$) for G_l . A modest differentiation was observed in the shear moduli values between the peripheral zone (PZ) and central gland (CG) but the differentiation was not significant ($p < 0.065$ for G_d and < 0.058 for G_l).

The receiver operating characteristic for each is shown in Figure 5. The overall sensitivity (specificity) was 0.69 (0.69) for G_d , 0.69 (0.69) for G_l , and 0.65 (0.65) for ADC in PZ; 0.65 (0.65) for G_d , 0.69 (0.66) for G_l , and 0.59 (0.60) for ADC in CG. The overall AUC was 0.75, 0.75 and 0.68 in PZ; and 0.69, 0.70 and 0.62 in CG, for G_d , G_l and ADC respectively. For the predictor model of the combined G_d , G_l and ADC case the overall sensitivity (specificity) was 0.75 (0.75) in PZ and 0.71 (0.71) in CG. The overall AUC for the combined case was 0.82 in PZ and 0.78 in CG.

The results of the six cases with multi-frequency data are shown in Figure 6. The following images are shown: the histology slides with marked tumors, the registered T2-weighted anatomy, the registered masks of the zones, registered G_d and G_l , Gamma as well as ADC. Even though the tumors are not particularly visible in the viscoelastic maps (or the ADC

image), the Gamma map seemed to highlight the small tumors. The mean Gamma in normal (cancer) was 1.6 ± 0.8 (1.1 ± 0.7) for G_d and 1.2 ± 0.9 (0.7 ± 0.7) for G_l in PZ; and 1.1 ± 0.9 (1.3 ± 0.8) for G_d and 0.8 ± 1.0 (1.2 ± 0.8) for G_l in CG. The differentiation of PZ from CG was not significant ($p < 0.2$) for Gamma.

The overall sensitivity (specificity) was 0.66 (0.69) for Gamma- G_d , and 0.68 (0.68) for Gamma- G_l in PZ; 0.65 (0.65) for Gamma- G_d and 0.66 (0.66) for Gamma- G_l in CG. The overall AUC was 0.69 and 0.70 in PZ; and 0.67 and 0.69 in CG, for Gamma- G_d and Gamma- G_l , respectively.

A correlation between the elasticity modulus and the fixation time was observed (especially in PZ) as shown in Figure 7. The longer the specimens were immersed in the fixation solution, the larger G_d and G_l became (approximately $+0.20$ kPa/h). This effect is more prominent for fixation time less than 80 hours (tumors size dependent). The shear moduli decreased in tumorous tissue as a function of fixation time at approximately 0.4 kPa/h. This effect resulted in reduced differentiation between normal and cancer. Similarly the ADC values for normal PZ tissue decreased at an approximate rate of -1.5×10^{-6} mm²/s/hr while cancer tissue increased at an approximate rate of 0.8×10^{-6} mm²/s/hr.

Discussion

General observations

The phantom study verified that the transducer did not interfere with the MRE experiments and did not overheat. The setup of the transducer alone took between 2 and 10 minutes. The transducer was capable of inducing waves with amplitudes beyond 200 μ m peak-to-peak at 350 Hz and less than 10% distortion. The advantage of having an electromechanically driver outside of the scanner is that gradient fields do not interact with the excitation coil which would potentially distort the mechanical waveform. This has previously limited the amount of current that would pass through the coil thus limiting excitation amplitude (10). The driver introduced can be easily adapted for other applications, for excitation frequencies of several kilohertz with sufficient amplitude even for stiff tissue with very low harmonic distortion. Also, this driver does not contain any ferromagnetic or permanent magnetic material that would pose a safety risk during setup and operation of the device (9). For the patient study, waves were clearly visible in all directions at multiple frequencies in *ex-vivo* prostate glands with amplitudes between 1 – 100 μ m for frequencies between 600 – 1000 Hz.

The reconstructed G_d and G_l images were registered to the histology slides with an error comparable to the in-plane resolution of the MRE experiments.

No significant delineation between PZ and CG was observed in the elastograms or the power law exponent, although some differentiation was observed for P06 and P16 in Figure 4.

The cancer detection rate based on G_d and G_l was modest and with many false positives but comparable to that of ADC with an approximate AUC of 0.62 – 0.75 . The combined MRE +DWI method improved the AUC to 0.78 – 0.82 , suggesting that complementary information from MRE and DWI may improve results for the *in-vivo* case, which remains to be proven.

Cancer tumors were observed in MRE and DWI images. We can make the following observations. Not all cancer tumors are stiffer than the normal tissue surrounding it (e.g., P05, P08, P10, P16, and P19). Some cancer could not be detected in any of the MR images T2, G_d , G_l , or ADC. In some cases, the tumors were observed in the shear modulus images but not in the ADC images (e.g., P17 and P06). Also not all tumors had lower ADC compared to the surrounding normal tissue (e.g., P17 and P05). Figure 8 shows how the patient data is distributed in a G_d versus ADC plot. The values are expressed as the differentiation between cancer and normal tissue expressed in percentage value. One would expect all cancer to have a higher shear modulus and lower ADC value. This corresponds to the second quadrant of this plot. However, as seen in Figure 8 only for a subset of tumors - the larger and more aggressive ones as graded by the Gleason score - fall in this quadrant. In fact, it appears that many tumors have lower shear modulus compared to the background. Also, three out of eight cases from our study show that ADC is in fact higher for tumors than normal tissue.

Stiffer structures within the prostate were mistaken for tumors. The effect of other confounders such as Benign Prostatic Hyperplasia (BPH), scarring from previous biopsy tracts, calcifications, or androgen therapy on the mechanical parameters is not known.

This is the first study to compare the power law behaviour ex-vivo prostate with histology marked with the Gleason score. However, the narrow frequency span limited the applicability of the power law which may be widened for future experiments and is typically several orders of magnitude in materials science studies.

One of the key limitations of this study is the significant effect of the fixation process on the prostate. The fixation alters the cells and tissue at a molecular level to increase their mechanical strength and stability. This increased strength and rigidity is desired to preserve the morphology of the sample for further analysis and grading. Due to institutional regulations, we were not able to perform our experiments on fresh prostate specimens. While our first results showed encouraging correlation between elasticity and histology, the effects of fixation were not well understood at the time experiments started. We found out later that, unfortunately, when the gland is soaked in the formalin fixation solution, the chemical solution does not infuse uniformly across the specimen. We observed that the tissue interface closer to the solution will stiffen up more than the tissue farther away from the solution. Also, the stiffening is time dependent which was not controlled in this work. The higher stiffness of the gland required higher excitation frequencies to be used in MRE. The ADC value was also affected by the fixation process in a spatially non-uniform manner as observed by McGrath *et al.* (7). Therefore, there are limitations on how these results can be translated to *in-vivo* applications. The fixation process may explain why the PZ appears stiffer (because the periphery of the prostate is closer to the chemical solution) than the CG in this work and others (7), while the opposite is the case in *in-vivo* tissue (19). Another consequence of the fixation process is the changed stiffness baseline for each case, depending on the time the gland was left in the formaldehyde solution (see Figure 8). This prevented meaningful ROC analysis of pooling all data sets since the stiffness baseline for each data set is different, thus only average values of sensitivity, specificity and AUC were reported.

Comparison to literature

In comparison to the literature, Dresner *et al.* (5) showed that the central gland is significantly stiffer compared to the peripheral zone, and that the elasticity increased as a function of mechanical excitation frequency. In addition, preliminary results suggested that prostate cancer might be delineated in the images. Similarly, in our study we found that elasticity did increase with frequency and showed that cancer would be distinguished from normal tissue in many cases. However, our evaluation of the stiffness of the PZ is not consistent with Dresner *et al.* (5) due to the effect of fixation.

In the second study by Dresner *et al.* (6), the fresh prostate specimens were assessed with MRE and compared to histology. These were manually matched with the corresponding anatomy images. The specimens were immersed in polysaccharide gel with the transducer rod inserted in the urethra. The excitation frequency was 350 Hz. The displacement field was encoded in all three spatial directions in a volume with a resolution of $0.31 \times 1.25 \times 3$ mm. For analysis, the specimens were split in 12 segments (A/P, R/L, Base/Mid/Apex) and only tumors larger than 4 mm were considered. Shear modulus values for cancer were calculated based on a manual ROI taken from the elasticity image. Shear modulus values for normal tissue were calculated based on the shear modulus of the whole segment. The elasticity value for normal tissue was 11.89 ± 3.72 kPa and 24.23 ± 7.62 kPa for cancerous tissue, with sensitivity of 89% and specificity of 90%. Although this study compared the results to histopathology, image registration and a voxel by voxel approach was not used. In our study, we found a much lower differentiation between cancer and normal (approximately between 3% and 240%) compared to Dresner *et al.* (approximately between 100% and 400%). The most likely explanation for this discrepancy is the effect of the fixation process, which may have drastically reduced the elasticity contrast between cancerous and healthy tissue. However, some of the discrepancy might also be related to (i) the voxel-to-voxel analysis approach used in this work versus the more optimistic the 12-segment analysis approach reported by (6), and (ii) the differences in excitation frequency (ours 600–1000 Hz vs. Dresner *et al.* 350 Hz).

In the study reported by McGrath *et al.* (7,8), the prostate was immersed in a gel prior to imaging. The displacement was encoded in a single direction over a volume with a resolution of $0.5 \times 0.5 \times 3$ mm. They showed that fixation significantly increases the elasticity value (4 ± 1 times) in a non-uniform manner. The increases correlated to fixation time (approximately 2.3 kPa/h) and the distance from the tissue edge formalin had diffused. This is consistent with the results we observed in our work. The stiffness dramatically increases in the first 24 hours. We had access to the specimen only after 24 hours which explains why we have different values for the slope of stiffness increase as a function of fixation time. Also, we found that the stiffness of the fixed *ex-vivo* specimen (at 800 Hz) had increased by 40 ± 30 times compared to the *in-vivo* gland (at 70 Hz). This value is much larger than reported by McGrath *et al.* We associated this difference to (i) experimental methods (ours is a 3D direct inversion of dynamic MRE while McGrath *et al.* used a 1D quasi-static MRE) and (ii) experimental conditions (ours compared *in-vivo* to *ex-vivo* after fixation while McGrath *et al.* compared *ex-vivo* before and after fixation). McGrath *et al.* also observed similar effects in the ADC values which decreased as a function of fixation time. The

changes were hypothesized to occur by the fixation-induced cross-linking, or methylene bridges, between neighboring proteins (20).

In conclusion, we described the development of a small-bore MRE system is described. The system has an excitation amplitude that is more than sufficient for stiff tissue even in high frequency (1kHz) MRE applications. By placing the electromagnetic driver outside the bore, no image artefacts due to the interaction of the gradient field with the exciter coils are present. The pre-compression is adjustable. The driver is safe to setup and operate because no ferromagnetic or permanent magnetic material is present in the driver. This setup was then used in an *ex-vivo* prostate cancer study where the results are compared to whole mount histopathology. The absolute shear modulus was able to diagnose cancer with an average AUC of 0.75 in PZ and 0.75 in CG. ADC and shear moduli may provide complementary information (combined AUC of 0.82 in PZ); in fact, in some cases, MRE performed better than ADC in distinguishing normal from cancerous tissue. Multi-frequency data acquisition did not improve the results, but again these results are preliminary. However, in light of effect of tissue fixation, the clinical results are inconclusive and more experiments are needed. A correlation was found between fixation-time of the prostate specimen to the stiffness of the tissue which may affect the diagnostic power results. It is thus recommended to continue the study with the examination performed before the fixation process. Furthermore, future studies should concentrate on a more targeted and detailed pathological analysis, as even with the confounding nature of the fixation process, it seems that prostate cancer detection based on tissue elasticity alone may not be as accurate as previously thought.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

1D/2D/3D	one/two/three dimensional
ADC	apparent diffusion coefficient
AUC	area under the curve
CG	central gland
DWI	diffusion weighted imaging
MRE	magnetic resonance elastography
MRI	magnetic resonance imaging

PC	prostate capsule
PZ	peripheral Zone
RF	radio frequency
ROC	receiver operating characteristic curve
SV	seminal vesicles
TE	echo time
TR	repetition time
TZ	transition zone

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Figure 1.

a) The custom-made mechanical transducer consists of a swiveling current-carrying coil that generates vibrations when the transducer is placed in the fringe field of the MR scanner. b) shows a photo of manufactured transducer as attached to the scanner outside the bore. c) The prostate specimen (with a rod inserted in the urethra) is placed on a cradle that is inserted into the RF coil. d) The transducer rod is a carbon-fiber tube that on one side is pressing on the apex of the prostate and on the other side is fastened to the transducer.

Figure 2.

A T2-weighted anatomical image of a phantom with spatially heterogeneous inclusions of different elasticity values was tested with the transducer. The resulting mechanical waves (350Hz) in the axial and coronal planes are as shown. The inclusions can be easily visualized in the reconstruction elasticity map. U_x (left-right), U_y (up-down), U_z (in and out of page)

Figure 3.

Typical MRE images acquired for an ex-vivo prostate: T2-weighted, 3D waves, reconstructed shear modulus G_d and the power-law exponent Gamma. The coronal (and axial for 600 Hz) projections of the displacement field in the three dimensions are shown. The experiment was repeated for different excitation frequencies (600, 800 and 1000Hz). The wave propagation is complex and present in all three axes. Ux (left-right), Uy (up-down), Uz (in and out of page)

Figure 4.

Sample results of shear moduli (G_d and G_1) and ADC as registered to histopathology. The tumors are visible in the viscoelastic maps even though in some cases it is not visible in the ADC maps.

Figure 5.

The receiver-operator-characteristic curve and the corresponding AUC is shown for each case for parameters G_d , G_i , ADC and combined (CA).

Figure 6.

Sample results of multi-frequency shear moduli (G_d and G_t) and power-law exponent Gamma as registered to histopathology. Even though the tumors are not particularly visible in the viscoelastic maps or the ADC image, the Gamma map seems to highlight the small Gleason 3+3 tumor (e.g. P09, P20).

Figure 7.

A plot of shear moduli G_d and ADC in the normal and cancer tissue in the peripheral zone as a function of the time the ex-vivo prostate gland was immersed in fixation solution.

Figure 8.
The differentiation between cancer and normal tissue for G_d versus ADC expressed as percentage.

Table 1

Clinical data for patients with biopsy confirmed cancer. (NPH=Nodular prostatic hyperplasia, PIN=Prostatic Intraepithelial neoplasia, PNI= Perineural invasion, SV= seminal vesicles, PUE=Prostatic Urethral Ectasia, BPU=Benign prostatic urothelium).

Case	Age (yr)	fixation (hrs)	Gland size (gr)	Gleason Score	Clinical Stage	# Tumors (size range mm)	Pathology
P01	61	24	48	4+3 (PZ)	pT2c	8 (4–20)	NPH, PIN
P04	71	84	61	3+4 (PZ)	pT2c	7 (2–9)	PNI, PIN, NPH, irregular nodule 10mm in left PZ
P05	59	72	32 (no SV)	3+3 (CG)	-	5 (3–8)	PIN, NPH, cyst (15mm) with calcification in TZ
P06	63	82	65	4+3 (PZ)	pT3b	5 (2–11)	PNI, PIN, NPH
P08	66	96	64	3+4 (PZ)	pT2c	3 (2–7)	PNI, PIN, NPH, irregular nodule 5mm right PZ base
P09	67	48	86	3+3 (CG)	pT2a	2 (3)	PIN, scars from biopsy, BPH with fibriadenoma-like pattern
P10	72	72	34	3+4 (PZ)	pT2c	5 (2–9)	prominent PIN, PIN, NPH, irregular nodule anterior, irregular mottling 10m PZ base
P11	69	24	40	3+4 (CG)	pT3a	15 (2–15)	PIN, NPH, irregular yellowish nodule left anterior PZ
P13	76	36	74	NA	pT2a	6 (3–7)	Pre-op androgen therapy, 8-month withdrawal
P16	70	72	72	3+4 (PZ)	-	11 (2–9)	PNI, PIN, NPH, scars from biopsy
P17	70	96	53	4+3 (PZ)	pT2a	4 (4–11)	PIN, PUE, scars from biopsy, NPH, cystic atrophy, Transurethral Curettage
P18	53	96	39	4+3 (PZ)	pT2c	6 (1–6)	PIN, NPH, BPU, 1 cm cyst
P19	53	72	53	4+4 (PZ)	pT3c	13 (2–18)	PNI, PIN, BPU, 1 cm utricular cyst,
P20	60	48	111	3+4 (PZ)	pT2c	22 (1–8)	PIN, NPH, BPU, scars from biopsy, benign cyst, compression of PZ by hyperplastic nodules of TZ

Table 2Average values for G_d , G_l and ADC in the PZ and CG zone for normal tissue and cancer. (p-value: <0.005

***; <0.05 **; <0.1 *)

Case	Zone	t-test (p-value)	G_d (kPa)		G_l (kPa)		ADC $\times 10^{-3}$ (mm ² /s)	
		G_d , G_l , ADC	Normal	Cancer	Normal	Cancer	Normal	Cancer
P01	PZ	***, ***, 0.88	30±5	102±17	24±5	84±9	-	-
	CG	***, ***, **	41±14	75±7	37±12	65±7	-	-
P04	PZ	***, ***, **	74±13	56±12	67±9	51±10	-	-
	CG	**, **, 0.77	55±21	89±6	54±20	84±4	-	-
P05	PZ	***, ***, ***	54±12	38±11	57±11	38±12	0.55±0.10	0.71±0.18
	CG	0.35, 0.53, **	32±12	33±10	29±10	30±11	0.75±0.16	0.78±0.15
P06	PZ	***, ***, 0.37	22±7	28±10	21±7	26±9	0.85±0.14	0.83±0.11
	CG	**, **, 0.65	16±8	22±3	14±7	20±3	0.93±0.16	0.91±0.08
P08	PZ	***, ***, ***	32±15	19±8	30±13	17±6	0.94±0.22	0.83±0.26
	CG	-	-	-	-	-	-	-
P10	PZ	***, ***, ***	94±59	80±42	79±48	62±29	-	-
	CG	-	-	-	-	-	-	-
P11	PZ	***, ***, ***	58±24	74±22	45±16	62±20	1.00±0.15	0.75±0.15
	CG	***, ***, ***	39±14	53±15	26±14	40±17	0.79±0.19	0.63±0.10
P16	PZ	***, ***, **	98±44	86±38	90±46	78±38	0.86±0.21	0.84±0.17
	CG	*, ***, ***	75±30	78±27	62±28	72±22	1.00±0.24	0.87±0.20
P17	PZ	***, ***, ***	68±24	75±18	54±18	63±17	0.83±0.19	0.95±0.15
	CG	0.28, 0.22, ***	84±48	79±20	72±42	66±14	0.83±0.26	1.05±0.18
P18	PZ	***, ***, *	74±32	100±30	62±27	77±26	0.82±0.18	0.79±0.09
	CG	0.11, *, 0.71	73±31	83±15	57±24	66±7	0.77±0.17	0.78±0.07
P19	PZ	***, ***, ***	122±50	90±29	98±44	73±23	0.78±0.14	0.51±0.11
	CG	*, 0.25, *	67±24	61±27	54±23	50±27	0.63±0.11	0.60±0.13
P20	PZ	**, **, 0.62	57±21	77±11	47±14	62±11	0.89±0.18	0.93±0.07
	CG	*, **, 0.17	64±21	56±15	52±18	43±15	0.78±0.20	0.73±0.16
Mean	PZ	-	65±25	69±21	56±21	58±17	0.84±0.17	0.79±0.14
	CG	-	55±22	63±14	46±20	54±13	0.81±0.19	0.79±0.13