Measuring the Effect of Formalin Fixation on Ex Vivo Tissue Material Properties using High Resolution 3D Quasi-Static MR Elastography at 7 Tesla for Improved Biomechanical Registration of Histopathology, and Correlation with the Effect of Fixation on T_1,T_2 and ADC

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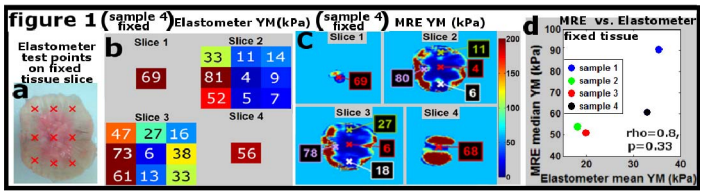
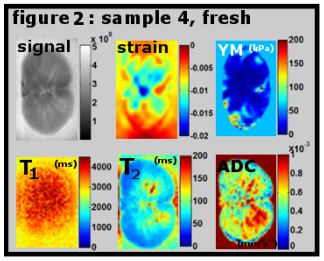
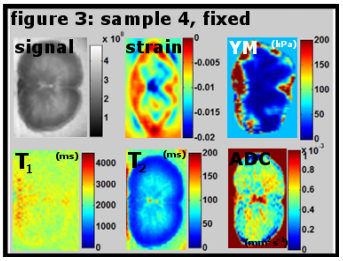
INTRODUCTION In targeted radiation cancer therapy, the correct identification of tumor boundaries is critical during planning, for accurate disease treatment and reduction of complications to normal tissue. Histology is considered the 'gold standard' for disease delineation. The tumor boundaries determined from imaging can be assessed retrospectively by correlation with histopathology, and this may lead to improved understanding of disease representation in imaging. When biomechanical registration (e.g. MORFEUS (1)) is used, knowledge is required of the ex vivo tissue material properties, and how these change with the pathology fixation process, i.e., fixation increases tissue stiffness (2), and as a function of distance from the edge of the sample. Pre-clinical tissue was used to validate a quasi-static MR elastography (MRE) method (2) with an iterative finite element modeling (FEM) solution, which was applied pre- and post-fixation. The results were validated using indentation testing. T_1, T_2 and ADC have been observed to decrease with fixation (3, 4). It was therefore investigated how changes in these parameters were related to the changes in stiffness as assessed by MRE, as these parameters may provide an alternative to MRE for assessment of the impact of fixation on material properties.

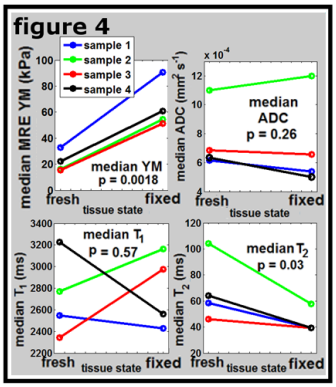
METHODS Ex vivo prostates (n = 4) from healthy dogs (3-5cm diameter) were embedded in a cube (7×7×7 cm^3) of gel (1.5% agarose, 4% gelatin) prior to imaging (2). The MRE compression device (2) consisted of a sample holder, a compression plate and mechanical piston, connected via an eccentric disk to a non-magnetic ultrasonic piezo-electric motor (USR60-E3N, Shinsei, Japan), providing compression at 1 Hz with maximum amplitude of 1.5 mm. The device was placed in the bore of a 7-T pre-clinical MRI scanner (70/30 BioSpec, Bruker, Ettlingen, Germany), where a quadrature volume resonator (15.5 cm inner diameter) was used for transmission and reception. Motion was tracked via the scanner pre-clinical respiration monitor (SA Instruments Inc., New York), which triggered the scanner to acquire during the compression phase. A Stimulated Echo (STEAM) sequence (5) was used, with a gradient duration τ = 1 ms and amplitude Gd = 40 mT/m which implied a displacement sensitivity ϕ_d of 3.4π. The mixing time T_m between the 2nd and 3rd STEAM 90° pulses was 200 ms. Acquisitions were made while the device was static, and again during motion, of 24 coronal slices of 3 mm thickness, with 0.5 by 0.5 mm in-plane resolution (160 × 160 matrix), with a TR = 1000 ms. Echo planar (EPI) readout was used: TE = 18 ms, NEX = 3, segments = 10. For the first 3 samples, 1 set of acquisitions was made (requiring 18 mins) providing a signal to noise (SNR): 100-200. For the 4th sample, imaging was repeated up to ~5 hrs (16 × 3 = 48 NEX), to obtain SNR~500. The strain maps were calculated from the motion and static image sets using the techniques described in (2), with the application of a Gaussian smoothing filter (σ = 5 pixels, width = 7 pixels) as a final step, to dampen noise. 3D maps of Young's modulus (YM) were generated from the strain data using an adaptation of the iterative FEM method of (6). In (6), to reduce the influence of noise, averaging over pre-defined tissue regions was employed. Here, a voxel-wise solution for the tissue was obtained by allowing averaging over the gel region alone. Via simulation, it was found that the mean percentage difference from ground-truth of the voxel-wise solution for SNR=500 was < 6 %. The samples were imaged fresh and again after overnight-fixation (<18 hrs) in 10% neutral buffered Formalin. An elastometer (7) was used to validate the MRE measures. Subsequent to imaging, the fixed samples were sliced into sections ~1 cm thick; the end slices were elastometer tested at one centre point and the mid slices tested at 9 points in a grid with approximate 1 cm spacing (figure 1a). MRE resulted in maps of relative YM between the tissue voxels and the uniform gel background. These were converted to kPa using the elastometer-measured gel YM (61±9 kPa). For visual comparison of the spatial distribution of fixation effects, for the high SNR acquisition of sample 4, the MRE YM maps were calculated at 0.5 × 0.5 mm resolution. However, for summary parameter comparison, to reduce the computation time for the FEM solution, the MRE data was first down-sampled 1.5×1.5 mm resolution. Median MRE YM values (less sensitive to errors in tissue boundary delineation than the mean) were compared with the mean elastometer values for fixed tissue. YM at (approximate) equivalent points were compared between indentation and MRE for the high resolution solution of sample 4. T_1, T_2 and ADC of the fresh and fixed tissue samples were quantified at consistent graphical prescription and voxel dimensions with the MRE acquisition (160 × 160 matrix, 0.5 by 0.5 mm in-plane resolution). T_1 and T_2 were measured using a RARE (Rapid Acquisition with Relaxation Enhancement) method adapted for quantification, with 5 echo times (TE = 7.5, 22.5, 37.5, 52.5 and 67.5 ms) and 6 TR values in the range: 1110-5554 ms. T_2 was quantified over the 5 TE, individually for each TR, and then averaged. T_1 quantification, based on saturation-recovery, used the 6 TRs at TE = 7.5 ms. The RARE factor was 2. The diffusion experiment to measure ADC used EPI and 3 orthogonal gradient directions: TR = 3500 ms; TE = 27 ms; segments = 8; NEX=1; b values = (0, 750 s/mm2); Gd= 266 mT/m. T_1, T_2 and ADC maps were generated according to the standard exponential functions in Bruker Paravision software (Paravision 5.0), and the results obtained were compared with the 3D material property maps of the tissue in both the fresh and fixed states. For sample 4, visual comparison was made (at 0.5×0.5 mm resolution), and, for all 4 samples, the median parameter values were compared. 1-tailed paired t-tests were used to compare the median of each parameter pre- and post-fixation.







RESULTS Figure 1b-c: YM values at corresponding points for fixed tissue (for sample 4) were comparable for MRE and indentation. MRE detects higher YM values (>200kPa) at the edges, where indentation was not feasible. Figure 1d: The summary YM values for all 4 fixed prostate samples appeared related, although not significantly correlated (Spearman). Figures 2 and 3: MRE YM increases in a non-uniform margin < 1 cm around the edge of the sample with fixation. In agreement with (3, 4), there are visible decreases in T_1, T_2 and ADC. For T_1 the decrease appears uniform across the sample, whereas for T_2 and ADC the decrease occurs predominantly at the edges. Figure 4: fixation caused a significant increase in median YM (~3-fold in all samples) and a significant decrease in median T_2, while T_1 and ADC did not demonstrate decreases in all samples. No significant correlations (Spearman) were found between any of the median parameters. The fresh tissue median MRE YM values (15-33 kPa) are comparable with those of in vivo canine prostate (in (8) Shear modulus = 5-6 kPa and, therefore, assuming a Poisson's ratio of 0.499, in vivo YM = 16-19 kPa).



DISCUSSION AND CONCLUSION The MRE method provides high resolution 3D material property information on fixation effects in ex vivo tissue samples, which are validated by indentation. T_2 was decreased by fixation in all samples, and may provide a marker for material property changes. Variable overall fixation effects between samples may be related to varying volume and fixation time (12-18 hrs). This MRE method will be used to generate population averages of material properties in fresh and fixed human prostate to improve registration of whole mount histology with imaging.

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