Independent testing of soft tissue visco-elasticity using indentation and rotary shear deformations

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Numerous techniques exist to measure the mechanical properties of soft tissues *in vivo*, such as mechanical stretching, indentation or shearing, as well as elastographic methods employing ultrasound or other imaging modes. Many groups have reported properties which do not necessarily correspond with each other due to differences in choice of technique, tissue model or other variations. This work deliberately makes use of the two independent modes of indentation and rotary shear, on the same material samples, employing similar modeling approximations, to attempt to determine the common, underlying material properties.

This paper introduces the ROSA-2 rotary shear instrument, and presents its mechanical characteristics, as well as presenting validation experiments that were performed to verify non-slip contact with tissue. Measurements made with it are compared with those acquired with the TeMPeST 1-D indentation instrument. Initial testing showed reasonably agreement when testing silicone gel samples, over a restricted range of frequencies. When testing bovine liver samples *in vitro* and porcine liver *in vivo*, significant discrepancies were found. The potential sources of these differences will be discussed, as will directions for ongoing work.

1. Introduction

The mechanical properties of soft tissues are of increasing interest for medical diagnosis and surgical simulation. In the former case, mechanical testing may aid in deciding whether or not to remove tissue when other tests are inconclusive or inconvenient. In the latter, it is essential that surgical trainees do not learn tasks incorrectly because of shortcomings of the training system. For example, a simulator with tissue stiffness much higher than real tissue could lead trainees to apply excessive forces when they first perform surgery on live patients. For both of these applications, detailed knowledge of the mechanical behavior and properties of living tissue is crucial.

Recently, numerous research groups have been actively developing techniques and instruments for *in vivo* determination of tissue properties. Purely mechanical testing may use indentation probes [1, 2, 3], tension testers [4], compression techniques [5], rotary shear applicators [6] or other deformations. Ultrasound elastography [7] and magnetic resonance elastography [8] combine mechanical deformation and measurement of resulting strain fields to extract elasticity data Results are beginning to become available for *in vivo* tests

(e.g. [1, 4]), but in many cases, the data obtained are not easily comparable, either because the tissue models employed or the testing domains (strain or frequency range) are not comparable, or merely because few tests are done on the same tissues. To begin validating the emerging data, a number of these obstacles must be overcome. The approach described here involves comparison testing of two instruments with independent testing modes on the same tissues, with overlapping ranges of applied strain and strain rates, and subsequent analysis of the results assuming the same constitutive model.

2. Methods and Instrumentation

The TeMPeST 1-D (Tissue Material Property Sampling Tool) indenter and the ROSA-2 Rotary Shear Applicator were used (see Figure 1). The TeMPeST 1-D will be briefly reviewed (see also [3]) and ROSA-2 function, calibration and validation tests will be described.

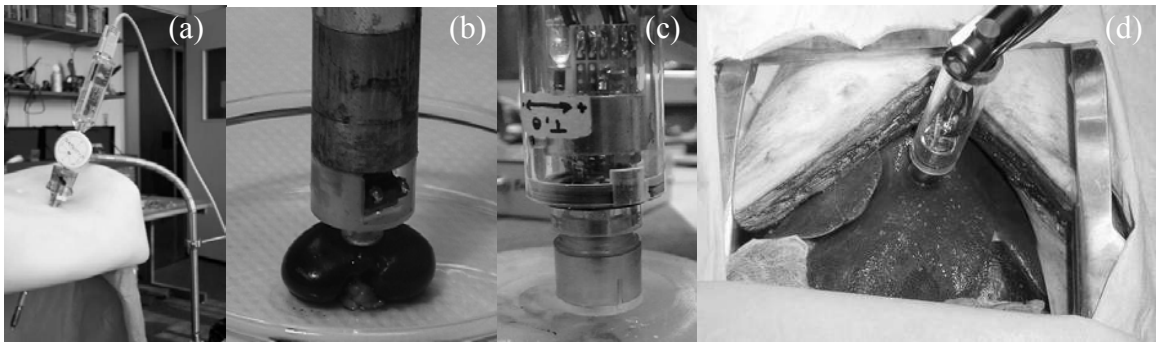

Figure 1: Instrumentation: (a) TeMPeST 1-D instrument passing through 12mm surgical port; (b) detail of indenter deforming rat kidney; (c) ROSA-2 with fixation ring; (d) ROSA-2 testing porcine liver *in vivo*.

2.1 TeMPeST 1-D

TeMPeST 1-D [3, 9] (Figure 1a,b) is a 12mm diameter minimally invasive instrument, designed to measure the compliance of solid organ tissues. A 5mm right circular punch vibrates the tissue while recording applied load and relative displacement. Mechanical bandwidth is approximately 80Hz when in contact with organ tissues; range of motion is 1mm; and forces up to 300mN can be exerted. It has previously measured the properties of porcine liver and spleen *in vivo*, rodent (rat) liver and kidney *in vitro* [10], and has been used in initial investigations of bovine, ovine and human vocal tissue samples *in vitro*.

2.2 ROSA-2

ROSA-2's (Figure 1c,d) 6mm right circular contact rotates relative to a concentric ring fixed to the tissue. Non-slip contact is maintained using a densely packed pin array or a tissue sealant/adhesive. A calibrated galvanometer exerts torque and rotations up to +/-15º are recorded with a non-contact, analog optical sensor with a resolution of ~0.004º. Rotor inertia and friction are smaller than in an earlier version [6], so higher frequency responses can be investigated. A recently implemented closed loop (CL) position controller provides a bandwidth of approximately 20Hz when in contact with materials similar in compliance to solid organ tissue, although it should be possible to extend this range.

Non-slip contact with the tissue is maintained with a densely packed annular array of fine needles (approx. 1500 steel needles of 100μm dia). or a disposable flat acrylic tip to

which a tissue adhesive is applied (see Figure 2). Band-Aid Liquid Bandage (a cyanoacrylate, Johnson & Johnson, Skillman, NJ) was used as the adhesive.

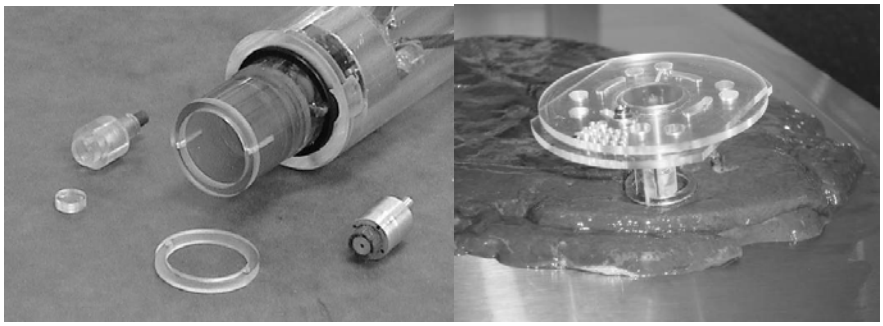

Figure 2: (Left) ROSA-2 rotary shear instrument showing (L to R): flat tip adhesive contact head (with contact tip); fixation ring (with contact ring); and needle array. (Right) *In vitro* test rig for non-slip and damage examination. Note holes in needle array for spinal needle alignment for injection of barium sulfate.

3. Experiments

A series of validation tests were conducted with ROSA-2 to study the non-slip characteristics of the two contact heads, damage-free tissue deformation limits, and finite element analysis (FEA) predictions of the region of influence of torsion testing. Following these tests, comparisons were made between the TeMPeST 1-D and ROSA-2 on silicone gel samples, bovine liver *in vitro*, and porcine liver and spleen *in vivo*.

3.1 Static testing for angular motion limits

A mock-up with the same contact geometry as ROSA-2 was constructed (see Figure 2), which could be used to apply known, fixed rotations to the tissue using either contact head. The mock-up was placed on or bonded to store-bought bovine liver samples, and the heads were rotated incrementally up to angular displacements of 90º for 100 seconds and returned to zero. Visual inspection and histological examination of the tissue was employed to determine the presence of damage and obvious slipping of the contact heads.

3.2 Fluoroscopic testing of region of influence

The contact heads and the static mock up were designed such that a 22-gauge spinal needle could be precisely placed adjacent to the contact zone (see Figure 3), allowing the injection of narrow lines of barium sulfate contrast medium into the parenchyma of the bovine liver tissue. Prepared samples were placed in the imaging field of a Siemens AG (Munich, Germany) Neurostar fluoroscope, and images were generated with rotations up to 90º. These early tests were for qualitative verification of FEA predictions, and to confirm the visual evaluations of slip conditions made previously.

3.3 Silicone gel tests: TeMPeST 1-D vs. ROSA-2

Two different samples[1] of two-part RTV silicone gel 6166 (GE Silicones, Waterford, NY), each 10cm in diameter and 12mm thick, were measured with both the TeMPeST 1-D and ROSA-2. Earlier TeMPeST results [3] were compared with new ROSA-2 tests, performed

[1] 30:70 and 40:60 mixtures of RTV6166 components A and B respectively, by volume. See [3] for additional measurements and comparison with parallel plate rheometry measurements.

under CL control with commanded mean and amplitude angular deformations of 15º and 5º respectively. The frequency range of chirp signal inputs was 0.1 to 100Hz.

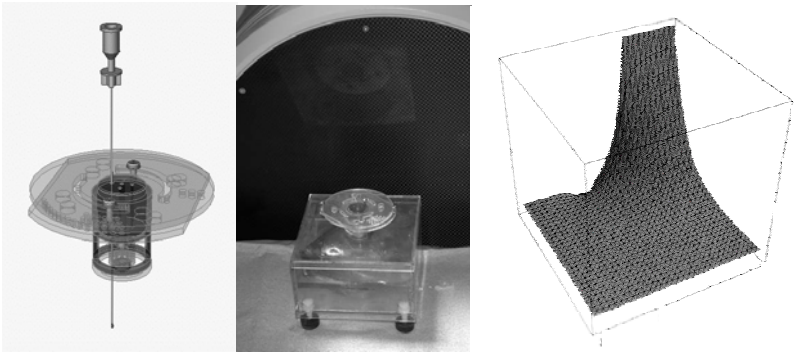

Figure 3: Static test rig showing spinal needle (left) and bovine liver sample in test apparatus in front of fluoroscopy detector (center). FEA model predicting rotation vs. radial position and depth (right)

3.4 Bovine liver tests in vitro: *TeMPeST 1-D vs. ROSA-2*

Bovine liver was measured with both instruments under open loop (OL) control at four different locations under the following conditions: ROSA-2 nominal torque preloads and amplitudes of up to 0.15 and 0.21mN·m, and frequencies from 0.0076 to 200Hz; TeMPeST 1-D force preloads and amplitudes of up to 72mN and 84mN, and the same frequencies.

3.5 Porcine liver and spleen in vivo: *TeMPeST 1-D vs. ROSA-2*

With appropriate approval, both instruments were tested on porcine liver and spleen *in vivo* (see Figure 1d). As previously described, TeMPeST 1-D testing was performed during 20 sec. periods of suspended ventilation, so that pulmonary action would not overwhelm the small deformations generated during normal indentation. OL force preload and amplitude for the TeMPeST 1-D were 35mN and 42mN respectively. ROSA-2 torque preload and amplitudes were 0.17 and 0.13 mN·m. Rotary action of the instrument relative to the fixed ring was less sensitive to breath motions, so sampling intervals lasted up to 131 sec.

4. Results & Discussion

4.1 Static testing for angular motion limits

The histology slides in Figure 4 show the untested surface, a region tested with the needle array and a sample after the adhesive disk had been removed. The needles caused no apparent damage, but removal of the adhesive disk destroyed the capsule, so any damage from testing alone is not apparent. However, since needle array testing caused no damage, adhesive disk damage, prior to removal, is unlikely.

4.2 Fluoroscopic examination of region of influence

Fluoroscopic imaging also qualitatively supports early FEA (see Figure 3), which indicates that the region of influence is approximately equal to the radius of the fixation ring. This can be seen in Figure 5, in which the depth of twisting of the barium sulfate lines is of the

same order as the distance between the center of the indenter and the fixation ring. Further, it can be seen that contact is maintained throughout the range of rotation.

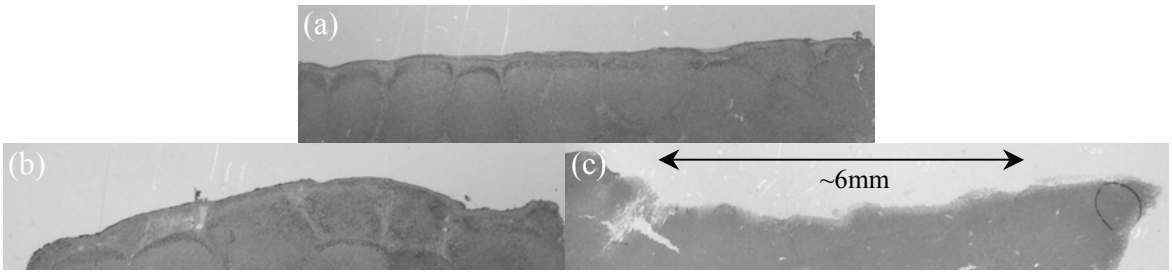

Figure 4: Histology slides of tissue (a) untested, (b) tested with needle array and (c) after removal of adhesive-bonded contact head. No visible damage in (b), but tissue surface is damaged post-removal.

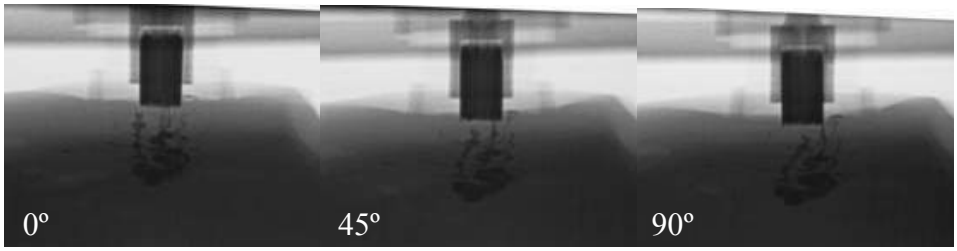

Figure 5: Sequence showing needle array on bovine liver sample. Three injections of barium sulfate visible as needle array is rotated from by 45º increments from 0º to 90º.

4.3 Silicone gel tests

Silicone gel tests show good agreement between the two instruments, at least over a restricted range of frequencies (approx. 0.5 to 10Hz, see Figure 6). Assumptions for the simple modeling scheme are: linear (visco-)elasticity, homogeneity, isotropy, incompressibility, small (infinitesimal) deformations and contact with a semi-infinite body. The ROSA-2 data further assume rotation neglecting the fixation ring. With these assumptions, compliance can be converted directly to elastic modulus values [11].

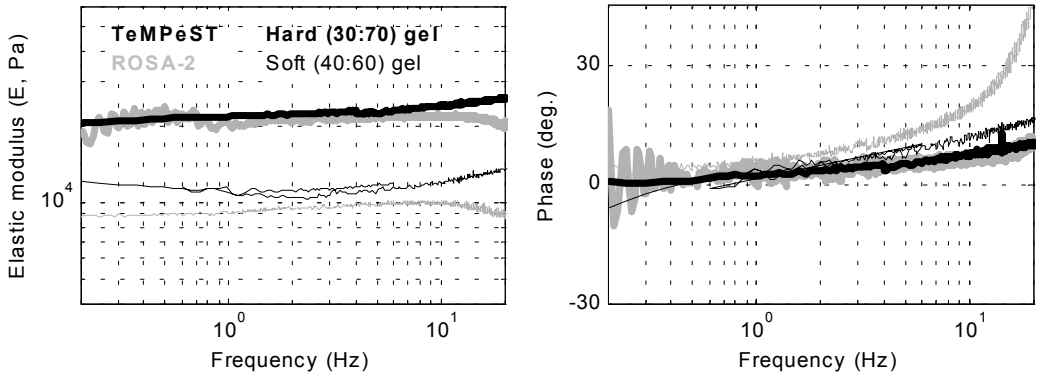

Figure 6: Silicone gel results. Elastic modulus in Pa (left) and phase angle (right).

4.4 *Bovine liver test* in vitro

Figure 7 shows significant discrepancies between the behavior of bovine liver samples as measured by the two instruments. At low frequencies, elastic behavior is observed (i.e. constant modulus and near zero phase angle), confirmed by both instruments. However the source of the nearly two order of magnitude shift in the curves is unclear, especially given the close agreements shown in the gel tests. At higher frequencies, a large contribution is

the inertial effect of the galvanometer, however this cannot account for the low frequency difference. Further analysis and deeper understanding of the measurements and instruments may provide insight to resolve this problem. One possibility is that the ROSA-2 testing reaches higher along the non-linear stress-strain response than TeMPeST 1-D tests. Future experiments will investigate smaller deformations to verify this explanation.

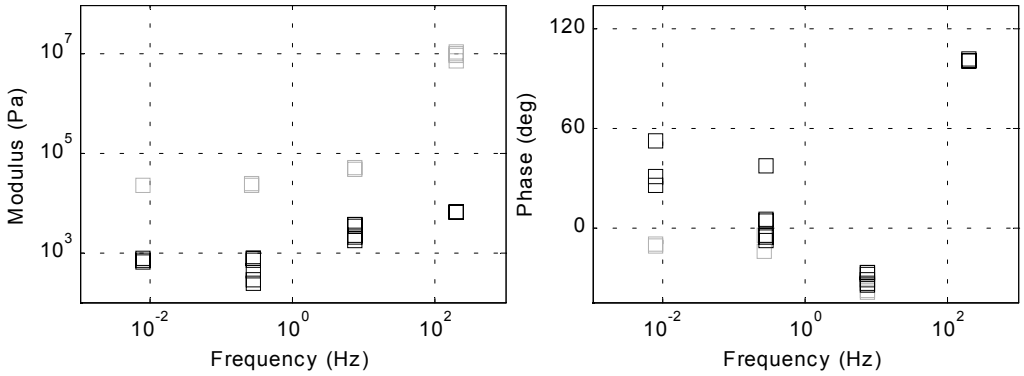

Figure 7: *in vitro* bovine liver results. ROSA-2 (grey), TeMPeST 1-D (black). Similar global behavior observed, but further analysis will be required to resolve discrepancies between instruments.

4.5 *Porcine liver test* in vivo

The *in vivo* testing shows similar results to the *in vitro* testing for ROSA-2, and also shows a much lower elasticity as measured by TeMPeST 1-D (Figure 8). Additional difficulties and noise in the data due to cardiac and pulmonary action make measurements less reliable.

Square wave testing *in vivo* demonstrated that the needle array can become clogged with continued use if it is not periodically rinsed to remove any deposits left behind by the fluids that "sweat" from the tissue itself. This was not observed in any testing with the adhesive disk.

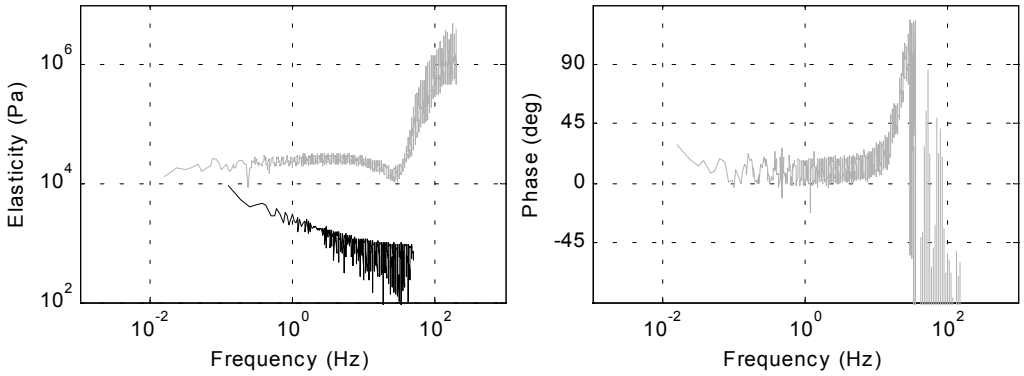

Figure 8: *in vivo* porcine liver results. ROSA-2 (grey) mean of 4 measurements; TeMPeST 1-D (black) n=12. Phase measurements for ROSA-2 unreliable above 50Hz; for TeMPeST 1-D corrupted by noise and omitted.

5. Conclusions and Future Work

This initial work in comparing rotary shear with indentation as soft tissue testing modes has shown agreement in the limited case of linearly elastic silicone gels, but more complex biological tissues result in widely differing elasticity measurements. Possible sources of the difference include the extremely simple tissue model employed, differing strain ranges and in the *in vivo* case, disturbances due to cardiac and pulmonary action.

The ROSA-2 evaluation indicates that non-slip contact can be maintained using either a needle array or adhesive disk, although use of the cyanoacrylate adhesive would not be suitable for survival animal tests, or human testing. An array of larger diameter needles might be less subject to the clogging problem and may be constructed in the future.

Future rotary shear instrumentation should include a torque sensor so that compliance can be measured at frequencies beyond the instrument-tissue system mechanical resonance. The next indentation probe should be insensitive to organ motion, and be able to measure absolute (not just relative) position. Finite element modeling based on real organ geometry will be pursued to better approximate the boundary conditions and finite deformations.

6. Acknowledgements

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