Keysight Technologies
In Vitro Complex Shear Modulus of Bovine Muscle Tissue (steak)
Application Note

Abstract

Dynamic instrumented indentation provides a way to measure the mechanical properties of soft biological tissue that is relevant, accurate, and easy. In this work, we measured the complex shear modulus of bovine muscle tissue, submerged in saline at bovine body temperature (38°C). Along the grain, the shear modulus was 24.4±13.9 kPa. Orthogonal to the grain, the shear modulus was 11.4±2.9 kPa. The loss factor was highly consistent and independent of testing direction: $\tan \delta = 0.34 \pm 0.035$.

Introduction

Being able to measure the mechanical properties is crucial to understanding soft living tissue. First, there is a reciprocal relationship between mechanical properties and function. That is, properties affect function, but function can also affect properties by means of adaptation. Thus, knowledge of mechanical properties leads directly to knowledge of function. Further, gel is often used as a substitute for living tissue [1]. In order to tailor the gel to mimic the tissue, one must first know the mechanical properties of the tissue. Thus, the purpose of this work was to demonstrate the use of instrumented indentation to measure the complex shear modulus of soft tissue under physiologically relevant conditions—submerged in saline at body temperature.

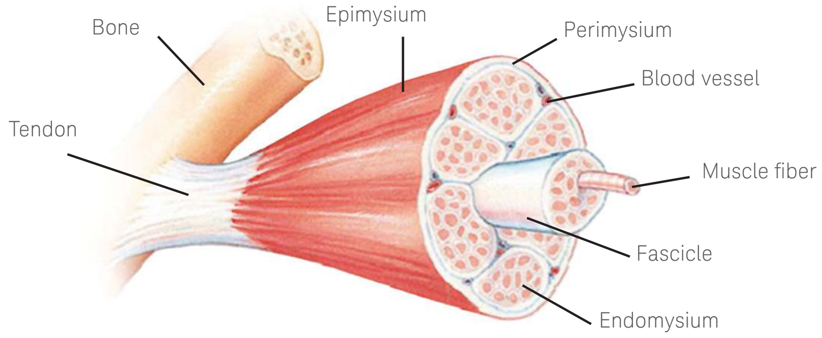

Figure 1. Schematic of skeletal muscle tissue (National Cancer Institute, public domain).

We chose bovine muscle tissue (steak) as an exemplary material. Figure 1 shows a schematic of skeletal muscle tissue. The “grain” of the steak runs in the long direction of the muscle tissue; this is the direction in which the muscle contracts and relaxes. A cut the grain of the steak reveals the same cross-section shown schematically in Figure 1. The proper term for a “grain” is the , which is a bundle of individual muscle cells or fibers. Given this structure of muscle tissue, we should expect the properties of the tissue to be anisotropic.

Dynamic instrumented indentation returns the stiffness (S) and damping ($C\omega$) of the contact at the prescribed frequency. Using Sneddon’s elastic contact theory[2] as later developed by Oliver, Pharr, and Brotzen[3] and Herbert et al.[4], the real and imaginary components of the complex shear modulus ($G^*=G'+iG''$) are calculated as

$$G' = S(1-\nu)/(2D), \text{ and}$$
$$G'' = C\omega(1-\nu)/(2D),$$

where ν is the Poisson's ratio of the material (assumed 0.5 for soft tissue) and D is the contact diameter (i.e. the diameter of the punch face). Traditionally, the shear loss modulus (G") is not reported as an absolute value, but in relation to the shear storage modulus. The loss factor, which characterizes the damping relative to stiffness, is calculated directly as

$$\tan\delta = G''/G' = C\omega/S.$$

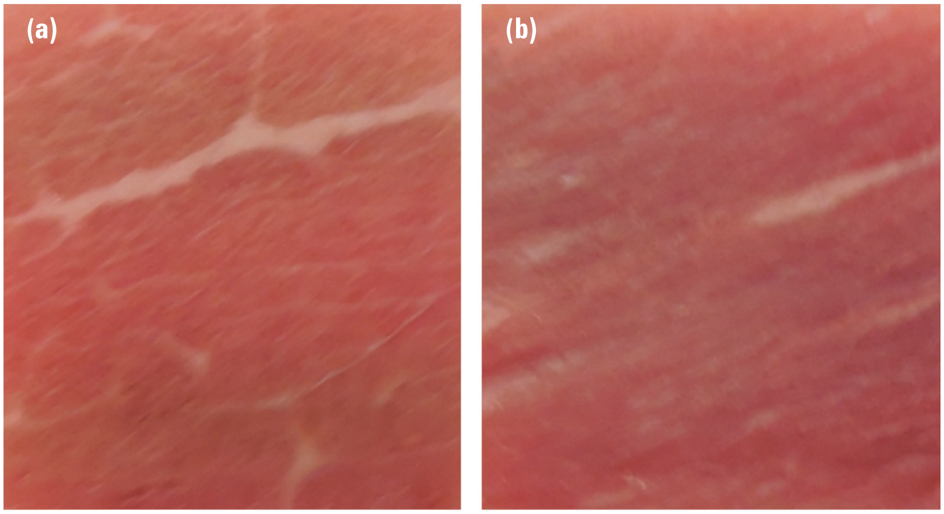

Figure 2. Steak samples as prepared for testing (a) cut across the grain, and (b) cut with the grain.

Experimental Method

The Keysight Technologies, Inc. G200 NanoIndenter was used for all testing. The system was configured with a DCM II actuator, the CSM option, and the hot-stage option. The CSM option allowed the superposition of an oscillating force, and the hot-stage option allowed the samples to be tested at body temperature. The DCM II actuator was fitted with a flat-ended cylindrical punch (D=101.1 µm) in order to generate a known and constant contact.

Two samples of steak were tested in this work: one cut across the grain (Figure 2a) and one cut with the grain (Figure 2b). Each kind of sample was prepared in the following way. The night before testing, the tissue was rough-cut and frozen. A disk (=8 mm, =8 mm) was cut from the frozen material and quickly adhered to the base of the sample well[1] using 5-minute epoxy. Once the epoxy had set, the well was filled with saline, and the sample holder was attached to the hot stage. Finally, a microscope slide was gently laid over the sample to keep it from drying while the sample equilibrated at the testing temperature (38°C). The equilibration time was about 30 minutes. Figure 3 shows a sample ready for testing.

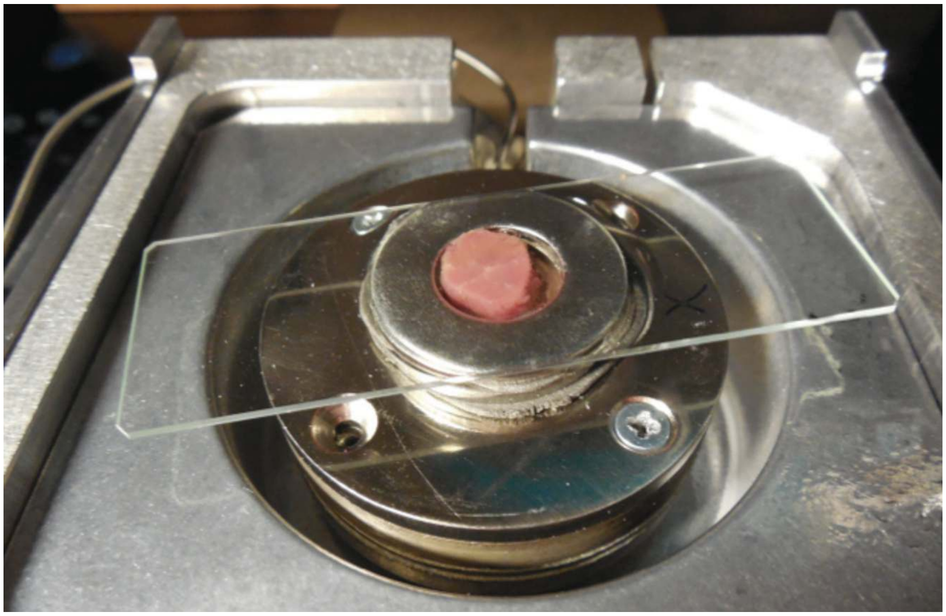

Figure 3. Steak sample, submerged and ready for testing at body temperature. Microscope slide was used to seal the sample and keep it moist until just prior to testing.

It should be noted that the testing direction is orthogonal to the plane of the cut. Thus, the sample which was cut across the grain (Figure 2a) was actually tested in the direction of the grain—that is, in the direction of the muscle fibers illustrated in Figure 1. The sample which was cut with the grain was actually tested perpendicular to the grain. Results are reported according to the direction of the *test*, not the cut.

The NanoSuite test method "G-Series DCM CSM Flat Punch Complex Modulus, Gel" was used for all testing. Each test with this method comprised the following steps:

1. Approach the surface, oscillating the indenter at its natural frequency (110Hz), until contact is detected,
2. Switch to the user-prescribed testing frequency (10Hz),
3. Apply pre-test compression (7µm),
4. Sense tissue stiffness and damping, and
5. Withdraw the indenter and move to the next test site.

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[1] In order to allow the samples to be submerged during testing, we used washers to build a "well" on the sample base plate for the hot-stage. Two washers, built up from the base plate with JB Weld SteelStik, provided sufficient depth (8mm).

With this method, twenty-five different sites were tested on each sample; individual test sites were separated by at least 400 µm. Tests were performed in red tissue only. Each test took about two minutes. In order to keep the samples from drying out, testing was paused after every 5 tests in order to add 1–2 drops of saline. Others have solved the problem of drying by integrating an intravenous drip [5].

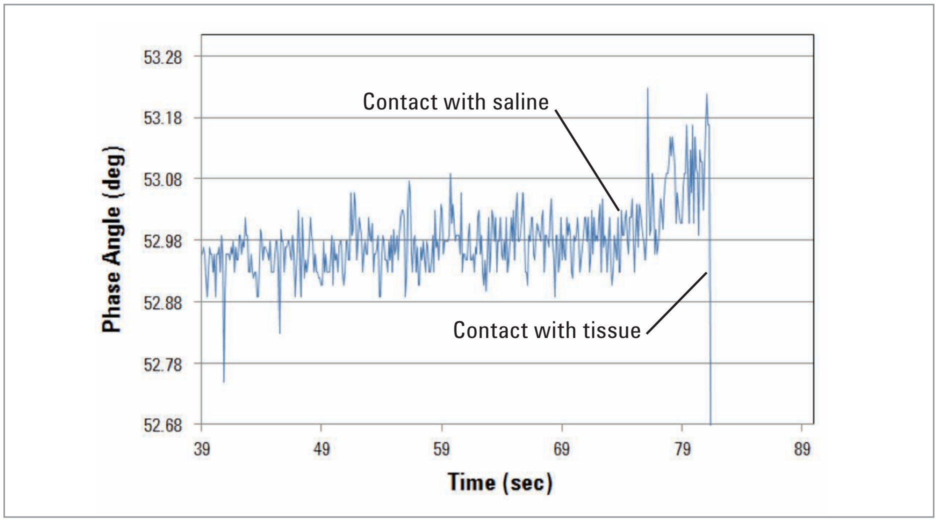

Figure 4. Signature of contact with saline and tissue.

Results and Discussion

Testing a sample submerged in saline poses no problems. Although the system indeed senses contact with the saline, the signature is different from that of contact with tissue (Figure 4). The attraction between the tip and the fluid causes a momentary decrease in the stiffness of the system. Thus, the interaction between the tip and the fluid manifests as a slight, but detectable, increase in the phase angle. Subsequent contact with the sample causes an increase in the stiffness of the system, thus manifesting as a sharp decrease in the phase angle. This extreme sensitivity in the phase angle is attained by oscillating the indenter at the natural frequency of the instrument (110 Hz) during the approach.

Figure 5 shows the results for shear modulus. The muscle tissue is significantly stiffer in the direction of the grain (G'=24.4 ± 13.9 kPa) than perpendicular to it (G'=11.4 ± 2.9 kPa). This is not surprising since the muscle tissue naturally acts in the direction of the grain to alternately exert and relax force.

The point-to-point variation in modulus is high, especially in the direction of the grain, but this is to be expected for biological materials. The observed variation is due to true point-to-point variation in properties, not measurement uncertainty [2]. Indeed, when we test uniform gels of comparable moduli, the point-to-point variation is quite low [6]. In the direction of the grain, this high degree of variation is likely due to the complex structure exposed by the cut across the grain (as illustrated by the cross-section of Figure 1). Perpendicular to the grain, the variation is smaller. Again, this is not surprising, because the structure is less complex in this direction.

The uniformity and consistency of the loss factor is quite surprising, however (Figure 6). The ability of the tissue to damp energy, as quantified by the loss factor is rather independent of the direction or location ($\tan\delta = 0.34 \pm 0.035$ for both directions). This surprising result begs for a phenomenological explanation. The value of the loss factor (0.34) means that at 10Hz, the capacity of the tissue to damp out energy is one third of its capacity to store energy elastically. This is physiologically realis-

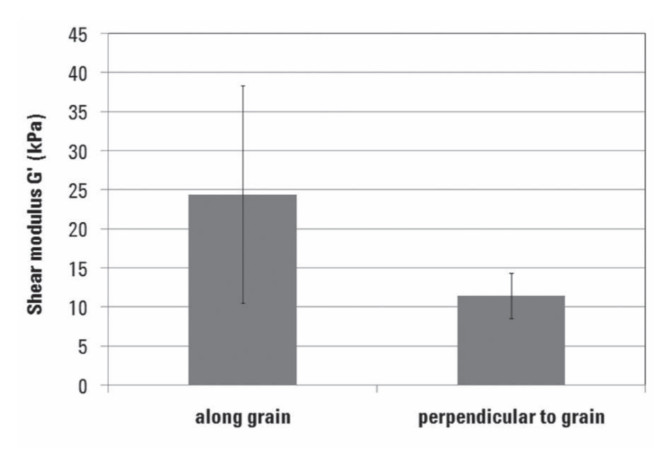

Figure 5. Shear modulus of bovine muscle tissue at 38°C, 10 Hz (N = 25).

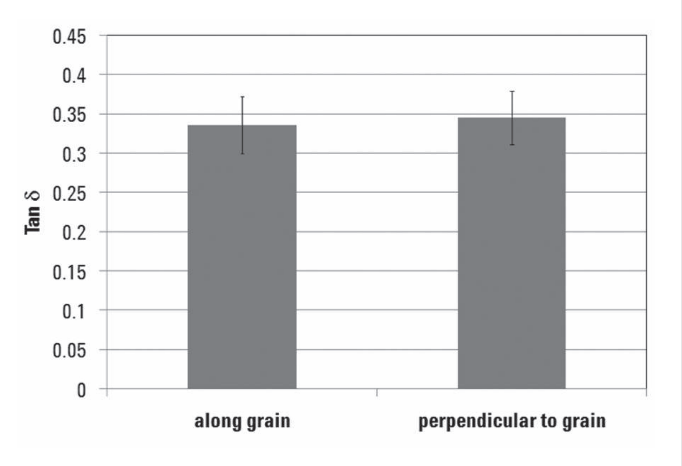

Figure 6. Loss factor of bovine muscle tissue at 38°C, 10 Hz (N = 25).

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2. At 10Hz, the uncertainty in the stiffnesses measured by the DCM II is less than 0.1N/m. In this work, the contact stiffnesses were 5–10N/m. Thus, we expect 1–2% variation due to measurement uncertainty.

tic and only measurable by keeping the sample submerged during testing.

Conclusions

The values for complex shear modulus measured in this work confirm the anisotropic nature of muscle tissue and provide a reliable basis for relating other comparable measurements. These extraordinary measurements are made possible by unique hardware and software features of the Keysight G200 NanoIndenter. The ability to oscillate the indenter makes the system sensitive to the slightest changes, and the software automatically responds to such changes to direct the progress of the experiment according to the experimenter's prescription. We anticipate that these techniques will be used by others to illuminate fundamental relationships between structure, history, and mechanical properties in biological tissues.

Acknowledgement

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