Nondestructive Ultrasonic Evaluation of Mechanical Properties of Agarose Gels.

1Walker, J.; 1McKee, A.; 1Berrilla, J.; 1Baskaran, H.; 1Mansour, J.; and 4Welter, J.F.
4Case Western Reserve University, Cleveland, OH. 1Authors contributed equally to this work.

Introduction:

Ideally, the mechanical properties of tissue-engineered cartilage should be known before it is implanted. Mechanical evaluation of tissue-engineered constructs requires destructive endpoint testing, for example, unconfined compression testing or indentation testing. This generally requires breaching the sterile bioreactor environment; thus constructs that have been tested are no longer useful for implantation. The purpose of this study was to evaluate the use of ultrasound as a non-contact, nondestructive means of determining Young’s modulus or the aggregate modulus of tissue engineered articular cartilage.

Methods:

A wide range of scaffold materials is used to support cartilage tissue engineering, including a host of hydrogel formulations. A simplified cell-free test system was used to demonstrate feasibility of the concept of using ultrasound-based non-destructive testing. Cylindrical, cell-free agarose hydrogels of varying percentages (1, 2, 5, and 10%) were used in this study to evaluate the experimental setup and methods. The gels were cast between glass plates using 12.7 mm diameter molds. As the percentage of agarose increases, the gels become subjectively much stiffer. To assess edge effects, each gel percentage was cast in thicknesses ranging from 1-10 mm.

A rigid fixture (Fig. 1) was designed to secure and align a 15 MHz submersible ultrasound transducer (Olympus NDT). For these studies, the test samples were placed in water on a 0.1% agarose bed. A Panametrics 5072PR pulser receiver and a digitizing oscilloscope (Pico-scope) were used for data acquisition.

One-dimensional elastic and porous elastic models were used to determine moduli from the ultrasonic data. Using the elastic model, Young’s modulus (E) was computed from

\[ c = \sqrt{\frac{E}{\rho}} \]

where \( c \) is the speed of sound, \( E \) is Young’s modulus, and \( \rho \) is the density of the hydrogel. In the porous elastic model it was assumed that the fluid and solid phases were incompressible materials, which are the assumptions typically applied when modeling cartilage. In this case, the speed of sound (c) is related to the aggregate modulus (H) by:

\[ c = \frac{\eta_f H}{\sqrt{\eta_s \rho_s + \eta_f \rho_f}} \]

where the subscripts s and f refer to the solid and fluid phases of the cartilage, \( \eta \) are the volume fractions of each phase, and \( \rho \) are the apparent densities, that is, the mass of each phase per total volume of hydrogel (Kumar, 2005). In both instances, the speed of sound (c) was computed using the known height of the sample, and the time difference between echoes from the sample’s upper and lower surfaces (Fig. 2).

Fig. 1: Ultrasound Alignment fixture

Fig. 2: Typical gated trace of the ultrasound echoes off the front and rear surfaces of an agarose gel.

Custom software was written in MatLab to evaluate the data.

Results:

Using the elastic model, a correlation between Young’s modulus and hydrogel concentration was found for the ultrasonic measurements. As expected the mechanical tests also revealed a positive correlation. The moduli determined from mechanical measurements were 6 orders of magnitude lower than those obtained from ultrasonic measurements, a trend that has also been observed by others in cartilage, and is likely due, at least in part, to the much higher strain rate in the ultrasound tests. Unfortunately, the correlation between ultrasound and mechanical moduli was weak using this simple model (not shown).

Using the porous elastic model, described above, a much stronger correlation between mechanical and ultrasound modulus was observed (R² on the order of .94) with only a 2-order of magnitude difference between the moduli, which again is consistent with the different strain rates. (Figure 3)

Discussion:

Our results suggest that ultrasound may be a useful tool for quality control of engineered cartilage. Unfortunately modeling using the simple elastic model seems unlikely to have much predictive value, but the more descriptive porous elastic model shows excellent correlation between ultrasound and mechanical tests of the same samples. These results suggest that the elastic model is incapable of providing meaningful estimates of Young’s modulus of gel-like materials with high water content, and in fact yields an estimate that more closely resembles the bulk modulus of water. This might be expected since in the one-dimensional model, the only difference between wave propagation in a fluid or solid is the replacement of the bulk modulus by Young’s modulus. In contrast, the porous elastic model includes both fluid and solid effects. The differences in magnitude between moduli determined from ultrasound and mechanically are due in part to the differences in strain rate and in part to the accuracy of the model that is applied.

For the simple elastic model, it was critical to know the sample dimensions to compute \( c \), which can be measured, and sample density \( \rho \) which had to be estimated but will likely remain close to 1 for the majority of the samples.

For the porous elastic model, an estimate of the volume fraction of the Agarose is required, which can be computed based on the dry weight of the agarose and the mass fraction of water in hydrated agarose. (Johnson, 1995). In cell-laden tissue engineering samples, these values are likely to change over time as the cells elaborate ECM of their own.

These baseline studies suggest that ultrasound could be useful to non-destructively monitor the progression of tissue maturation in live tissue-engineered cartilage. In particular the results using a porous elastic model seem promising. The advantage of these speed-of-sound based models is that the entire specimen thickness is sampled, unlike other approaches that are based only on the reflections and thus only sample a small amount of the tissue near the surface. The system is adaptable for use in our bioreactor system.

References: