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The nonlinear relationship between speed of sound and compression in articular cartilage: Measurements and modeling

Joseph M. Mansour^{a,+}, Mostafa Motavalli^{*,b,c,+}, Jay Bensusan^a, Ming Li^{d,e}, Seunghee Margevicius^d, Jean F. Welter^{b,c}

^aDepartment of Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH, USA

^bCase Center for Multimodal Evaluation of Engineered Cartilage, Case Western Reserve University, Cleveland, OH, USA

^cDepartment of Biology, Case Western Reserve University, Cleveland, OH, USA

^dDepartment of Population and Quantitative Health Sciences, Case Western Reserve University, Cleveland, OH, USA

^eCurrent Address: Division of Biostatistics, Department of Preventive Medicine, USC Biostatistics Core, USC Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA

Abstract

We measured speed of sound in bovine articular cartilage as a function of compressive strain. Using techniques we developed, it was possible to apply strain starting from the unstrained, full height of a sample. Our measurements showed that speed of sound was not a monotonic function of strain as reported in earlier investigations. Speed increased with increasing strain over a range of lower strains. It reached a maximum, and then decreased as the strain increased further. These results were corroborated using a model of wave propagation in deformable porous materials. Using this model, we also established conditions under which a maximum in the speed would exist for samples in compression. Our measurements and analysis resolve the conflicting results reported in previous studies.

*Corresponding author. Tel.: +1 216 368 0619, sxm591@case.edu (M. Motavalli).

⁺Both authors contributed equally to this study

Joseph M. Mansour*: Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing – Original Draft, Supervision, Project administration. **Mostafa Motavalli***: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – Review & editing, Visualization, Supervision. **Jay Bensusan**: Investigation, Data curation, Review & editing. **Ming Li**: Formal analysis, Review & editing. **Seunghee Margevicius**: Formal analysis, Review & editing. **Jean F. Welter**: Conceptualization, Resources, Review & editing, Project administration.

* Both authors contributed equally to this study

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Keywords

Ultrasound; speed of sound; compressive strain; articular cartilage; porous-elastic wave modeling

1. Introduction

“Cartilage” encompasses a range of soft connective tissues that are classified as articular (hyaline cartilage), fibrocartilage, or elastic (Chang and Martin, 2019). They are found throughout the body, for example, in freely moveable synovial joints, nose, trachea, meniscus, sternum, intervertebral disc, and ear. Articular cartilage, meniscus and intervertebral discs, among other cartilaginous tissues, function as load-bearing tissues, which has motivated investigations of the mechanical properties and relationships between structure and function in these tissues (Buckley et al., 2008; Chen and Broom, 1999; Motavalli et al., 2014; Nickien et al., 2013; Shirazi and Shirazi-Adl, 2008). Mechanical properties such as aggregate modulus, Poisson’s ratio and permeability have typically been determined using tests that include confined and unconfined compression, and indentation. Using these tests, the properties of normal native tissue, osteoarthritic cartilage, tissue engineered cartilage, and tissues treated with enzymes to determine the effects of specific components on mechanical properties have been cataloged (Armstrong et al., 1984; Best et al., 1994; Buckley et al., 2010; Bursa et al., 1999; Fithian et al., 1990; Mow et al., 1980). In addition to purely mechanical tests, acoustic methods are also used to characterize cartilage. Using ultrasound, acoustic properties such as speed of sound, reflection coefficient, attenuation, and apparent integrated backscatter have been used to characterize a range of tissue states (Bridal et al., 1997; Cherin et al., 1998; Gelse et al., 2010; Hattori et al., 2004; Kiviranta et al., 2009). Speed of sound, which is a relatively simple measurement, correlates with properties such as dynamic modulus and Young’s modulus (Saarakkala et al., 2004).

Speed of sound is an essential component in ultrasound elastography that is used clinically to characterize internal, depth-dependent displacement fields and hence depth-dependent variations in stiffness (McCredie et al., 2009; Righetti et al., 2007; Zheng et al., 2002). In this application, displacement is found using the time shift of internal acoustic reflections due to externally applied deformation and speed of sound. Assuming constant speed of sound can give rise to large error in the predicted displacements. The error in assuming speed of sound is constant has been reviewed in detail in other publications (Lotjonen et al., 2009). Our interest in measuring speed of sound as a function of deformation stems from earlier work where we have shown that, using this measurement, it is possible to predict mechanical properties of hydrogels (Mansour et al., 2014). Our long-term goal is to extend this work on hydrogels to tissue engineered cartilage while it is developing in a bioreactor.

Previous investigations have shown that speed of sound in cartilage is a function of deformation (Ling et al., 2007; Lotjonen et al., 2009; Nieminen et al., 2007). Although speed of sound is known to change with deformation, current studies offer differing results. Some investigations have shown that speed of sound decreases when cartilage is compressed, while others have shown that speed increases under compression. The purpose of this investigating was to evaluate speed of sound in articular cartilage as a function of strain. We

also investigated, using a mathematical model for wave propagation in porous-elastic materials, the influence of physical properties on relationships between speed of sound and deformation, and to corroborate the observed acoustic behavior of cartilage under compressive strain with a simulation model.

2. Materials and Methods

2.1 Sample preparation

A bovine knee was obtained from a local butcher shop. Osteochondral samples, 6 mm in diameter were cut from the femoral groove using a diamond coring tool under continuous irrigation with phosphate buffered saline (PBS). Location of the samples on the bovine distal femur taken at least 5 mm away from the osteoarthritic lesion are shown in Fig. S1. A sample was processed for Toluidine blue histology (sample No. 13, Fig. S2). Samples were frozen in PBS until use. On the day of test, samples were defrosted, and allowed to equilibrate for at least 30 minutes. A full-thickness disk of cartilage was then cut from the underlying bone using a razor blade. Only samples where the articular surface was as parallel as possible to the cartilage-bone interface were used. Speed of sound was measured in nine samples.

2.2 Speed of sound measurements

We used an apparatus for measuring the speed of sound as a function of strain that was similar to that used in previous investigations (Mansour et al., 2014) (Fig. 1). It consisted of a frame (Minitec, Victor, NY) that supported a sample container, and a micrometer controlled linear slide (Daedal, Parker Hannifin, Charlotte, NC) to which an ultrasound transducer and load cell were coaxially attached. In addition to supporting a sample, the bottom of the container acted as a reflector for the ultrasound signals. The 10 N load cell (Omegadyne LCMFD-10N) and associated controller (DP25B-S-A) were obtained from Omega Engineering (Stamford, CT). All acoustic measurements were made using a 20 MHz delay line transducer with a 4.7 mm diameter (model VM208, Olympus NDT, Waltham, MA). A JSR DPR500 pulser-receiver with a RP-L2 remote pulser was used in transmit/receive mode. The pulse repeat frequency, signal energy, damping, and gain were 200 Hz, 12.4 μ J, 44 Ohms, and 0 db. No hardware filtering was used. A Keysight Infiniivision MSOX3054T 500 MHz oscilloscope (Keysight Technologies, Santa Rosa, CA) was used for data acquisition. The oscilloscope was triggered by the pulser-receiver external trigger signal, the sampling rate was 5 GS/s, and for each sample 64 reflections were averaged to reduce random noise.

Prior to measuring speed of sound, the height of a sample was determined using a two-part approach similar to that described in a previous publication (Mansour et al., 2014). First, the “zero” position for the micrometer was established at the bottom of the stainless steel PBS bath. Zero was set when the leading edge of the reflection from the delay line aligned with the leading edge of the reflection from the bottom of the sample container and simultaneously there was a jump in the force sensed by the loadcell. At this point the face of the external delay line just contacted the bottom of the sample container, and the micrometer position was set to zero. A sample was then placed in the container and fully submerged in

PBS. Contact of the delay line with a sample was set at the point where the reflection from the surface of the sample merged into that from the face of the transducer, which also coincided with a 2 gram force on the sample. The micrometer reading at that point was the sample height, h .

Compressive displacement increments of $50 \pm 5 \mu\text{m}$ were then applied manually to a sample over a period of five seconds. Ultrasound data was acquired immediately after each increment, and time of flight of the reflections from the top and bottom surfaces of a sample (Δt) was measured using the cursors on the oscilloscope. Speed of sound, c , was then calculated from

$$c = \frac{2h}{\Delta t} \quad (1)$$

The change in speed of sound with respect to that at zero strain was found from

$$\Delta c = \frac{c(\epsilon) - c(\epsilon = 0)}{c(\epsilon = 0)} \quad (2)$$

and was compared with previous investigations (Ling et al., 2007; Lotjonen et al., 2009). In these investigations, a prestrain was applied and that position was used as the zero for subsequent applied strains. To compare their results to ours, we added a prestrain, 10% for (Lotjonen et al., 2009), and 5% for (Ling et al., 2007), to the strains reported in those investigations.

2.3 Modeling wave propagation

Measured speed of sound was compared with the speed of sound predicted by a model of one-dimensional wave propagation in deformable porous media with incompressible solid and fluid phases. (Deboer et al., 1993)

$$c^2 = \frac{\eta_f^2}{\eta_f^2 \rho_{b,s} + \eta_s^2 \rho_{b,f}} H = g(\eta_s, \eta_f, \rho_{b,s}, \rho_{b,f}; \epsilon) H \quad (3)$$

In this expression, subscripts s and f refer to the solid and fluid constituents, H is the aggregate (one dimensional) modulus, the η_i are volume fractions, and $\rho_{b,i}$ are bulk densities ($i = s$ for solid phase and f for the fluid phase). Expressions for the η_i and $\rho_{b,i}$ were developed in terms of the compressive displacement, d , of a material element.

The total volume of the element is $V_T = (h_0 - d)A$, where h_0 is the initial height of the element, and A is an arbitrary surface area, which does not affect the one dimensional analysis (Fig. 2). Since the volume of the solid in the material element does not change with deformation, its volume fraction (V_s/V_T) was computed as

$$\eta_s = \frac{\eta_{s0} h_0 A}{(h_0 - d)A} = \frac{\eta_{s0} h_0}{(h_0 - d)} \quad (4)$$

where η_{s0} is the solid volume fraction before deformation. Using this expression, and the fact that the sum of the volume fractions is one, the volume fraction of the fluid, (V_f/V_T) was found using

$$\eta_f = 1 - \eta_s = 1 - \frac{\eta_{s0}h_0}{(h_0 - d)} \quad (5)$$

Bulk densities are the mass of a constituent per total volume of the material element. Since the mass of the solid is not a function of displacement, we found its bulk density as

$$\rho_{b,s} = \frac{m_s}{(h_0 - d)A} \quad (6)$$

Rather than estimating m_s , the explicit dependence on mass was eliminated by using the true density, $\rho_s = m_s/V_s$. Therefore, the bulk density was computed from

$$\rho_s \frac{V_s}{V_T} = \frac{m_s}{V_T} = \rho_{b,s} \quad (7)$$

Similarly, the bulk density of the fluid was

$$\rho_f \frac{V_f}{V_T} = \frac{m_f}{V_T} = \rho_{b,f} \quad (8)$$

Using volume fractions and bulk densities (Equations (4), (5), (7) and (8)), the function g in Equation (3) was computed. Note that g depends only on deformation. It does not depend on speed of sound or modulus.

Following measurement of the speed of sound, solid volume fraction and solid bulk density were determined for four of the samples from lyophilization. These samples were frozen (-20°C), placed in a Petri dish and were lyophilized over 48 hours using an AdVantage BenchTop Freeze-Dryer (SP Scientific, Warminster, PA). For the other five samples, the average of the measured values of the solid volume fractions and solid bulk densities were used. These were the initial values for the solid volume fraction, and solid bulk density. Values for the modulus H were determined by fitting Equation (3) to measured speed of sound (c_m). From Equation (3),

$$\frac{c_m^2}{g} = H(\epsilon) \quad (9)$$

Since the functional form for H is unknown, three possible candidates were evaluated: a constant value, H_0 , a linear function of strain (ϵ)

$$H_l = H_{0l} + k_l \epsilon \quad (10)$$

or a H quadratic function of strain

$$H_q = H_{0q} + k_{ql}\epsilon + k_{qs}\epsilon^2 \quad (11)$$

The resulting function $H(\epsilon)$ was then fit to linear or quadratic expressions to determine the coefficients H_{0l} , H_{0q} , k_l , k_{ql} and k_{qs} . Predicted values for speed (c_p) were then found (Equation (3)) as

$$c_p = \sqrt{gH} \quad (12)$$

Differences between the measured and predicted speed of sound were evaluated quantitatively by the square root of the sum of the squared deviations.

Conditions under which a maximum in the speed of sound would be found under compressive strain were also investigated. From Equation (3), the strain corresponding to an extreme value of the speed was found from

$$\frac{dc}{d\epsilon} = \frac{1}{2\sqrt{gH}} \left(g \frac{dH}{d\epsilon} + H \frac{dg}{d\epsilon} \right) = 0 \quad (13)$$

For this analysis we used $g = g_0 + g_l\epsilon$, and H was as given by Equation (10). Therefore,

$$\epsilon = - \frac{g_0 k_l + H_{0l} g_l}{2 k_l g_l} \quad (14)$$

is the strain at which a maximum in the speed will be found. Since the slope of g is inherently negative (as will be shown in the Results), and in this analysis the sign convention for the applied compressive strain is positive, a maximum in the speed of sound at a positive compressive strain will exist if

$$|g_0 k_l| \geq |H_{0l} g_l| \quad (15)$$

All calculations were performed in Excel.

2.4 Statistical Analysis

The data set contained nine samples, and each sample had multiple measures of strain and speed of sound (Tables S1 – S2, Supplemental Data). For the nonlinear relationship between strain and speed of sound, a mixed effect polynomial regression model with a quadratic term in strain was used. This model included a random effect of intercept for samples. All tests were two-sided, and p-values less than 0.05 were considered statistically significant. SAS version 9.4 (Cary, NC) was used for analyses.

3. Results

3.1 Speed of sound

For all samples our measurements show that speed of sound is not a monotonic function of strain (Fig. 3). At lower strains, speed increases as a function of strain. It peaks between approximately 6% and 30% strain, whereupon the speed decreases with increasing strain. The percent decrease in the speed is greater at higher strains, than the increase of the speed at lower strains (Fig. 4). The change in speed from two other investigations is also shown (Ling et al., 2007; Lotjonen et al., 2009). These were offset to include the prestrains used in those investigations.

3.2 Modeling wave propagation

Solid volume fraction and bulk density determined by lyophilization for four of the samples (7, 14, 26 and 27) are shown in Table 1, along with average values that were used for the other five samples.

As the strain increases, g decreases monotonically (Fig. 5, Table 3). Although g is a nonlinear function of strain, for the volume fractions typical of cartilage, it is well fit by a linear model ($R^2 = 1.00$ for all samples). For the case where H is constant, the predicted speed must also decrease monotonically with increasing strain. In this case, the change in speed is due solely to the strain-dependent volume fractions and bulk densities, i.e., the function g . Therefore, considering a constant value for H to predict the speed of sound with respect to strain was not pursued, since our measurements showed that speed of sound did not decrease monotonically with respect to applied strain. In contrast, if H is a linear or quadratic function of strain (Equations (10) and (11)), speed of sound predicted by Equation (3) increases with strain at lower strains followed by decreasing values at higher strains, as in our measured data (Figs. 6a, 7a), subject to the conditions imposed by Equation (15). For some samples, the linear and quadratic models for H give essentially the same function for speed vs. strain, and have small deviations from the measured speed (Fig. 6a, Table 2). In these cases, the nonlinearity in H is small (Fig. 6b, Table 3). In other samples, the speed predicted using a quadratic model for the modulus more closely simulates the measured data than the linear model (Fig. 7a, Table 2). In these cases, the effect of the nonlinearity in H is stronger (Fig. 7b, Table 3).

In three samples, (13, 26 and 27), speed, predicted using Equation (3) with $H(\epsilon)$ as a linear function of strain, decreases monotonically. These result are consistent with Equation (14) that shows the maximum speed is at a negative and strain, and Equation (15) where the inequality is not satisfied.

3.3 Statistical analysis

Statistical analysis shows that there is a significant non-linear relationship between strain and speed of sound ($p=0.0267$ for strain and <0.0001 for strain squared). In the mixed effects quadratic polynomial regression model, the fixed effects are strain and strain squared. The linear term (strain) describes the linear trend with speed of sound across the data, and the quadratic term of strain squared shows a directional pattern of curvature. The random

effect allows the regression model that is to fit for an individual sample to differ in intercept from other individual samples.

In Table 4, the coefficient for strain and strain squared are 0.0017 and -0.00008 , respectively, indicating that the curve rises initially and then falls. The scatter plot presents individual sample's data points and the predicted mean line with 95% confidence interval band (Fig. 8).

4. Discussion

Developing methods for the nondestructive evaluation of tissue engineered cartilage, while it is maturing in a bioreactor, is a long term goal in our laboratory. Several issues support the need for such evaluations. One is that the quality of tissue engineered cartilage is highly variable and unpredictable. Thus, weeks of personnel time, and materials, may be invested in culturing a piece of tissue only to find, after weeks in a bioreactor, that it is unsuitable for implantation. However, by monitoring development, inferior tissue could be identified and abandoned before its prescribed end point. In contrast, tissue that is suitable for implantation could be identified, which makes this evaluation a factor for establishing release criteria.

We are using ultrasound for evaluating tissue development in a bioreactor since it is nondestructive, it can interrogate the full thickness of a cartilage construct, and measurements can be performed rapidly. In addition, we have established the feasibility of using ultrasound to evaluate the mechanical properties of tissue in a bioreactor using agarose hydrogels as a surrogates for cartilage (Mansour et al., 2014). Our approach is based on a statistical model developed from a data "training set". The sole input to this predictive model is speed of sound as a function of compressive displacement for each sample. Average or other characteristic values for speed of sound versus deformation cannot be used in this model. Thus, this measurement is of central importance to our work, and was the motivation for this investigation.

Variation in the speed of sound with deformation is not a new concept, nor is it limited to biological materials. For example, in elastic materials formulations based on finite deformation and third order terms in the strain energy have been shown to predict measured changes in speed of sound with strain (Egle and Bray, 1976; Hughes and Kelly, 1953; Murnaghan, 1951).

In articular cartilage, one study showed speed increasing as a function of strain, while several others have shown that speed decreases as a function of strain (Ling et al., 2007; Lotjonen et al., 2009; Nieminen et al., 2007). Here, we have shown that the speed of sound in native bovine articular cartilage initially increases as a function of strain, before decreasing as the applied compressive strain increases further. Thus, our results encompass the results of previous investigations.

There are several possible reasons for the differences between our results and those in the literature. These include the amount of prestrain, the time(s) after deformation at which the speed of sound was measured, and the joint from which the cartilage was obtained.

Prior to measuring speed of sound, Ling et al., applied a 0.05 mm displacement to their samples (Ling et al., 2007). After allowing samples to relax for 15 minutes, that position was assigned as the zero point for subsequent displacements. Since sample height was not specified, the prestrain is not known. Therefore, direct comparisons to other investigations, or ours, are only approximate. Although the exact “zero” for strain is not known, it seems reasonable to assume that it was not greater than 5% (0.05 mm displacement on a 1 mm thick sample). At lower strain, our results and those of Ling et al., showed that the speed of sound increased (Fig. 4). However, the increase of the speed in Ling et al., was greater than that in our investigation. This may be due to testing cartilage from different sites: Ling et al., used bovine patella cartilage and we used bovine cartilage from the distal femur.

Several other investigations that used bovine cartilage applied a 10% prestrain (Lotjonen et al., 2009; Nieminen et al., 2007). In this case, the change in speed vs. strain was found to decrease, similar to what we found at higher strains (Fig. 4).

The porous elastic model we used to simulate speed of sound was based on modeling the fluid and solid constituents as incompressible, assumptions that are commonly used to model cartilage (Deboer et al., 1993; Mow et al., 1980). This model (Equation (3)) makes no assumptions or imposes any conditions on the functional relationship between speed and deformation. It was then possible to compute volume fractions and bulk densities of each phase as a function of strain, and therefore $g(\eta_s, \eta_f, \rho_{b,s}, \rho_{b,f}; \epsilon)$. We showed that this term decreases with strain, and therefore, with a constant value for H , the predicted speed of sound must also decrease with strain, which is not in accordance with our measurements.

However, speed of sound found using linear or quadratic models for H more closely simulated our experimental data. That is, the predicted speed increases at lower strains, peaks and then decreases at higher strains. Modeling H as a quadratic function of strain resulted in predicted speed of sound that was as good or better than modeling H as a linear function of strain. This suggests that H should be modeled as a quadratic. In those cases where linear and quadratic models for H gave similar fits to the measured speed, the coefficient of the squared term was relatively small, and H was essentially a linear function of strain. In addition to the observation that a quadratic form for H gave the best prediction of the measured data, it is also supported by the results of repeated stress relaxation measurement that have shown that the equilibrium stress is not a linear function of total strain (Ling et al., 2007; Toyra et al., 1999). Statistical analysis also confirms that, overall, the relationship between strain and speed of sound is nonlinear.

An entirely different approach that was used to explain changes in speed of sound with deformation in cartilage was based on the hypothesis that change in speed is a function of changes in collagen fiber orientation and changes void ratio due to deformation, (Lotjonen et al., 2009; Nieminen et al., 2007). Using fiber orientation and void ratio computed from a finite element model of deformation, this relationship was calibrated against measured speed, in a subset of samples, and then applied to samples not used in the calibration. It was shown that this approach accurately predicted that speed will decrease with strain. However, as any compressive strain will result in a change in fiber orientation and fluid volume fraction, as formulated, this approach will always predict that speed will decrease as a

function of strain. Therefore, this approach cannot predict the initial increase and subsequent decrease in speed as we have measured.

In this investigation we used native bovine articular cartilage, as a proof of concept. Ultimately we intend to use this approach, validated by an analytical model, to evaluate mechanical properties of tissue engineered cartilage, its relationship to structure and composition, and development while in a bioreactor. In future studies, this can be used as metric to compare the properties of tissue engineered to native tissue. Additionally, this approach is not limited to native tissue or articular cartilage. In a broader range of studies, this measurement approach, and the analytical model can be explored to investigate the properties of porous-elastic materials.

5. Conclusions

In summary, we have shown that speed of sound in cartilage initially increases as it is subjected to compressive strain, but as the strain increases further, the speed of sound decreases. Measuring speed of sound over a wide range of strain, starting without prestrain, resolves the conflicting results reported in previous studies. Furthermore, we showed that our experimental results are consistent with predictions of a mathematical model for wave propagation in saturated porous-elastic materials (Figs. 6, 7).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1 -

Close-up of the apparatus used to measure speed of sound of an articular cartilage sample under compressive strain. A sample is sandwiched between the ultrasound (US) delay line transducer (top) and the surface of the stainless-steel PBS bath. It is compressed using the Z-axis micrometer.

Fig. 2 - Porous-elastic material element used to compute volume fractions and bulk densities for each displacement increment d . The initial height of the element is h_0 . The surface area A is arbitrary and does not affect these calculations.

Figure 3 -

Speed of sound as a function of strain measured in our laboratory. Our measurements show that speed increases at lower strains, peaks, and then decreases at higher strains.

Fig. 4 -

Change in speed of sound relative to the value at zero strain. Data from Ling et al., and Lotjonen et al., is offset to account for the prestrain applied in those investigations. Data was not recorded for sample 19 at zero strain. For clarity, data between 0 and 0.1 strains are replotted and included in the Supplementary Data (Fig. S3).

Fig. 5 -

The function g , that depends only on volume fractions and bulk densities, is inversely related to strain.

Fig. 6a -

Measured speed of sound and simulated values using a linear or quadratic model for the one dimensional modulus, H (sample 9). In this case both models for H predict speed of sound that closely follows the measured data (Table 2). b - The one dimensional modulus, H, found using Equation (11) and linear or quadratic models. In this case the effect of the quadratic term in H is small compared with that in shown in Figure 7b.

Fig. 7a -

Measured speed of sound and simulated values using a linear or quadratic model for the one dimensional modulus ((sample 10)). In this case the quadratic model for H results in a much closer fit to the measured data than does the linear mode (Table 2). b - The one dimensional modulus, H, found using Equation (11) and linear or quadratic models. In this case the effect of the quadratic term in H is much greater than for the sample shown in Figure 6b.

Fig. 8 –.
Predicted mean for all data with 95% confidence interval, and individual data points for each sample.

Table 1-

Volume fractions and weights determined by lyophilization

Sample	Wet Weight (g)	Dry Weight (g)	Solid Volume Fraction	Fluid Volume Fraction
7	0.0404	0.00646	0.155	0.845
14	0.0427	0.00982	0.178	0.822
26	0.0538	0.0145	0.0814	0.919
27	0.0411	0.00861	0.164	0.836

Table 2-

Square root of the sum of the squared deviations(SSQD) between the measured speed of sound and that predicted using a linear or quadratic model for the modulus. In all cases, the quadratic model produced less deviation and hence better fit to the measured speed (lower values of SSQD).

Sample	Linear $H(e)$	Quadratic $H(e)$
7	54.2	43.3
9	18.1	10.1
10	178.6	27.8
13	264.5	38.8
14	94.4	21.9
15	16.0	15.7
19	80.5	41.5
26	51.1	5.5
27	20.6	13.2

Table 3-

Coefficients for the linear and quadratic models for H, and the function g.

	$H_q = H_{0q} + k_{ql}e + k_{qs}e^2$			$H_l = H_{0l} + k_{ll}e$		$g = g_0 + g_l e$	
Sample	H_{0q}	k_{ql}	k_{qs}	H_{0l}	k_{ll}	g_0	g_l
7	4.92E+08	1.01E+09	2.01E+08	4.84E+08	1.11E+09	5.28E-03	-6.22E-03
9	7.34E+08	1.09E+09	-3.11E+08	7.41E+08	9.67E+08	4.06E-03	-4.73E-03
10	7.76E+08	9.90E+08	2.32E+09	6.63E+08	2.30E+09	4.06E-03	-4.74E-03
13	6.44E+08	6.44E+08	-1.05E+09	6.66E+08	6.66E+08	4.06E-03	-4.73E-03
14	9.13E+08	1.19E+09	1.71E+09	8.52E+08	2.00E+09	3.52E-03	-4.12E-03
15	6.34E+08	1.04E+09	3.51E+07	6.33E+08	1.05E+09	4.06E-03	-4.72E-03
19	4.83E+08	2.25E+09	-2.41E+09	6.16E+08	9.70E+08	4.06E-03	-4.74E-03
26	9.45E+08	1.57E+09	-2.84E+09	9.80E+08	7.67E+08	2.88E-03	-2.95E-03
27	3.07E+09	2.72E+08	-5.27E+09	3.15E+09	-1.43E+09	3.94E-03	-4.54E-03

Table 4-

Mixed effects quadratic polynomial regression model of the relationship between speed of sound and strain

	Beta	95% CI	P-value
Strain	0.00168	(0.0002, 0.00317)	0.0267
Strain ²	-0.00008	(-0.00011, -0.00005)	<.0001