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## Nonlinear elastic behavior of phantom materials for elastography

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### Abstract

The development of phantom materials for elasticity imaging is reported in this paper. These materials were specifically designed to provide nonlinear stress/strain relationship that can be controlled independently of the small strain shear modulus of the material. The materials are mixtures of agar and gelatin gels. Oil droplet dispersions in these materials provide further control of the small strain shear modulus and the nonlinear parameter of the material. Since these materials are mostly water, they are assumed to be incompressible under typical experimental conditions in elasticity imaging. The Veronda–Westman model for strain energy density provided a good fit to all materials used in this study. Materials with a constant gelatin concentration (3.0% dry weight) but varying agar concentration (0.6–2.8% dry weight) demonstrated the same power law relationship between elastic modulus and agar concentration found for pure agar ( $1.89 \pm 0.02$ ), consistent with percolation theory, and provided a consistent nonlinearity parameter of  $4.5 \pm 0.3$ . The insights provided by this study will form the basis for stable elastography phantoms with stiffness and nonlinear stress/strain relationships in the background that differ from those in the target.

### 1. Introduction

Quasi-static ultrasound elastography is an imaging technique that has been developing rapidly over the last 15 years. More challenging procedures of obtaining and analyzing data are being pursued. As image formation algorithms become faster and more efficient, thus improving the available technology, higher strains are being applied during the echo signal acquisition (Jiang and Hall 2007). The most common method for obtaining elastography images (elastograms or strain images) is to correlate echo signals acquired before and after uniaxial deformation (Ophir *et al* 1999, Hall 2003).

Most of strain imaging involves deformations of up to 5% which is a region of nearly linear behavior. However, when the compression becomes large enough most human tissues present a significantly nonlinear stress/strain relationship without a permanent change in microscopic structure (Humphrey and Delange 2004, Fung 1993). Breast tumors for instance can exhibit different nonlinear behaviors (Krouskop *et al* 1998). Attempts to determine which hyperelastic model is capable of fitting breast tissues stress/strain data have been reported (O'Hagan and Samani 2009). However, the relationship between biological structures and nonlinear stress/strain behavior is not completely understood. It is known that the stress/strain relationship for all networks composed of semiflexible filaments is

nonlinear and in the case of stiffer filaments such as collagen, they always stiffen for strains lower than 20% (Storm *et al* 2005).

Approaches that take advantage of the strain stiffening properties to improve elastography image interpretation have been reported (Erkamp *et al* 2004a, Nitta and Shiina 2002, Oberai *et al* 2009).

In order to further develop methods for estimating tissues' nonlinear parameters with elasticity imaging techniques, the nonlinear stress/strain properties of phantom materials must be better understood. Agar and gelatin are common materials used in manufacturing phantoms to be used in strain imaging (Hall *et al* 1997). The ultrasonic properties and elastic long-term stability of these materials are well established. It has been shown that agar/gelatin mixtures provide a way of obtaining reasonably stable material properties (Madsen *et al* 2005); moreover, regarding agar/gelatin mixtures, when the inclusion and background have the same gelatin concentration, the gelatin provides a good bond between the inclusion and background and stable geometry, i.e. the inclusion does not change volume due to osmosis (Madsen *et al* 2005). Therefore, all of the agar/gelatin materials produced in this work had the same gelatin concentration in the agar/gelatin component.

Agar is a well-known material for its nonlinear stress/strain relationship (Hall *et al* 1997, Nakakura *et al* 1992, figure 6), while for gelatin the stress/strain relationship is nearly linear (Hall *et al* 1997). Erkamp *et al* (2004b) adopted the Mooney–Rivlin strain energy density to obtain a nonlinear model to fit the force versus displacement curves for both materials. Although the Mooney–Rivlin model provides a good fit to the stress/strain data, this approach has a large number of parameters (usually more than 3) to be determined. In order to overcome this problem, the Veronda–Westmann model (Veronda and Westmann 1970, Gokhale *et al* 2008), which has only two parameters (nonlinearity parameter and shear modulus at zero strain), was used here and has been applied in biological nonlinear modulus reconstructions (Oberai *et al* 2009). This model assumes uniaxial deformation of an isotropic and incompressible material. It is common to assume that aqueous gel phantom materials are incompressible or nearly incompressible soft solids for the experimental conditions typical of elastography (see, for example, Hall *et al* (1997), Erkamp *et al* (2004b), Gennisson *et al* (2007)). Although the nonlinear elastic behavior of agar gels and gelatin gels has been reported, the nonlinear elastic behavior has not been described for mixtures of agar and gelatin. Madsen *et al* (2005) reported nonlinearity of agar/gelatin materials at higher agar concentrations and low strain ranges (see table 5 of Madsen *et al* (2005)), but strain values did not exceed 6%. Further, the stability of heterogeneous configurations of agar/gelatin mixtures containing oil droplets has been reported (Madsen *et al* 2005), but the nonlinear stress/strain relationship for these materials has not.

We report an investigation into the nonlinear stress/strain relationships of agar/gelatin mixtures and how that elastic behavior is affected by varying the volume fraction of oil in the dispersion. These materials are being designed for future use in elastography phantoms where heterogeneous configurations (e.g. spherical targets in a uniform background) are anticipated. Thus, materials should be designed with long-term stability in mind. A report on the time-dependent elastic behavior of these materials will follow.

## 2. Materials and methods

Agar is a gel-forming polysaccharide with a sugar skeleton. These gels are normally classified as a crosslinked network meaning the gel network is formed during congealing from an aqueous solution. In the solution state, the agar molecules are randomly distributed as a coil structure. After gelation, the collagen becomes a three-dimensional network of

double helix fibrils. A good review of these features and a model reporting how they relate to agar rheological characteristics can be found in Labropoulos *et al* (2002).

The double helix concentration is assumed to be proportional to the agar concentration. This assumption suggests that the agar elastic modulus can be analyzed with percolation theory (Fujii *et al* 2000) as described below.

Mixtures of agar and gelatin were previously analyzed (Clark *et al* 1983, Horiuchi and Sugiyama 1987) where it was observed that a granular pattern of agar gel (mainly) was formed into the gelatin matrix. The granule sizes were in the magnitude order of micrometers. The explanation given is that since the gelation temperature of agar is higher than that of gelatin, the agar becomes a gel before the gelatin.

From those analyses, several questions are easily raised: (1) What is the nonlinear stress/strain behavior of agar/gelatin mixtures compared to plain agar and plain gelatin? (2) How does the nonlinear behavior of agar/gelatin mixtures depend on agar dry weight concentrations? (3) Is the stiffness of agar/gelatin mixtures for different agar concentrations consistent with percolation theory? (4) If oil dispersions are made with agar/gelatin mixtures, how does the oil concentration affect the nonlinear elastic properties of the final material? The studies reported here were designed to answer these questions.

## 2.1. Nonlinear behavior

Some polymer gels as well as tissues are normally assumed to be hyperelastic. There are several constitutive relations which were developed to model the nonlinear behavior of this kind of material. These models are normally described based on the strain energy  $W$ s, which is the potential energy stored in the material due to a deformation. In a simple system,  $W$  equals the force acting on an object times its displacement. In a continuum system, the stored potential energy (see, for example, Humphrey and Delange (2004), Fung (1993)) can be represented in the same way. However, in this work, we consider a strain energy density  $W$ :

$$W = \int_{\lambda_i=1}^{\lambda} \sigma_i d\lambda_i, \quad (1)$$

where  $\lambda$  is the applied Cauchy stress,  $\lambda_i = 1 + \varepsilon_i$  ( $\varepsilon_i$  is the strain) and  $W$  has units of energy per unit volume or force per unit area. Since strain energy density does not depend on body motions, it is normally represented as a function of the strain invariants. Those parameters appear from the deformation gradient ( $F$ ) and the right Cauchy–Green tensor ( $C$ ), respectively, given by equations (2) and (3). For a better understanding of the following definitions (equations (2)–(7)), the reader is referred to Humphrey and Delange (2004, pp 271–87)

$$F = \begin{bmatrix} \frac{\partial x}{\partial X} & \frac{\partial x}{\partial Y} & \frac{\partial x}{\partial Z} \\ \frac{\partial y}{\partial X} & \frac{\partial y}{\partial Y} & \frac{\partial y}{\partial Z} \\ \frac{\partial z}{\partial X} & \frac{\partial z}{\partial Y} & \frac{\partial z}{\partial Z} \end{bmatrix} = \begin{bmatrix} \lambda_1 & \lambda_{12} & \lambda_{13} \\ \lambda_{21} & \lambda_2 & \lambda_{23} \\ \lambda_{31} & \lambda_{32} & \lambda_3 \end{bmatrix} \quad (2)$$

$$C = F^T F. \quad (3)$$

The deformation gradient was represented in terms of Cartesian coordinates where  $(x, y, z)$  locates a point of interest in the current configuration and  $(X, Y, Z)$  locates the same point in its original configuration. Assuming uniaxial deformations, the three strain invariants ( $I_1, I_2, I_3$ ) derived from  $C$  can be represented by the following equations:

$$\begin{aligned}
 I_1 &= \text{trace}(C) = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \\
 I_2 &= \frac{1}{2} \{ [\text{trace}(C)]^2 - \text{trace}(C^2) \} = \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_1^2 \lambda_3^2 \\
 I_3 &= \det(C) = \lambda_1^2 \lambda_2^2 \lambda_3^2.
 \end{aligned}
 \tag{4}$$

The second Piola–Kirchhoff stress tensor  $S$  is one definition of stress that is useful in nonlinear elasticity constitutive formulations:

$$S = \frac{\partial W}{\partial E_s} = 2 \frac{\partial W}{\partial C}, \tag{5}$$

where  $E_s = \frac{1}{2}(F^T F - I)$  is the Green–Lagrange strain tensor. The Cauchy stress can be represented as a function of  $S$ :

$$\sigma = \frac{1}{\det F} F \cdot S \cdot F^T. \tag{6}$$

All the materials in this paper are considered to be incompressible which means that the volume  $V$  is constant when the material is deformed, and therefore  $\det F = \lambda_1 \lambda_2 \lambda_3 = 1$ . Knowing that the samples used in the mechanical tests were cylinders and considering that the volume did not change during the test, it is reasonable to state that (equations (2) and (4) and Humphrey and Delange (2004, p 283))  $\lambda_1 = \lambda$  and  $\lambda_2 = \lambda_3 = 1/\sqrt{\lambda}$ . Therefore, the invariants become  $I_1 = \lambda^2 + 2/\lambda$ ,  $I_2 = 2\lambda + 1/\lambda^2$  and  $I_3 = 1$ .  $\lambda$  is named as the stretch ratio and can be understood as  $\lambda = (L + \Delta L)/L$  where  $L$  is the sample length (thickness) at zero deformation stress,  $L + \Delta L$  is the length at some nonzero deformation stress and  $\Delta L$  is the deformation. Using those relations and equations (3)–(6), the stress can be written in terms of  $\lambda$  and  $W$ :

$$\sigma = 2 \left[ \frac{\partial W}{\partial I_1} \left( \lambda^2 - \frac{1}{\lambda} \right) + \frac{\partial W}{\partial I_2} \left( \lambda - \frac{1}{\lambda^2} \right) \right]. \tag{7}$$

In this case the area of the sample used to calculate the stress must be corrected for the fact that the (incompressible) material has a deformation-dependent surface area. The experimental methods employed in this study involve a controlled deformation in the axial direction, so the corrected instantaneous cross-sectional area ( $A_c$ ) can be written as

$$A_c = \frac{AL}{L + \Delta L}, \tag{8}$$

where  $A$  is the initial cross-sectional area.

**2.1.1. The Veronda–Westman model**—Several semi-empirical representations of  $W$  exist (Fung 1993). These models are normally classified into polynomial or exponential models. The polynomial model of many terms, known as Mooney–Rivlin, is suitable for a large range of materials. However, having many terms (and parameters to estimate) in the hyperelastic strain model is a drawback when creating materials to make a specific sort of phantom.

The Veronda–Westman model (Veronda and Westmann 1970) for hyperelastic strain is one example of the exponential approach. This model is a good tradeoff between accuracy and complexity and also has an advantage of having only two independent parameters to be determined.  $W$  for this model can be written as

$$W = \mu_0 \left[ \frac{e^{\gamma(I_1 - 3)}}{\gamma} - \frac{1}{2}(I_2 - 3) \right], \quad (9)$$

where  $\mu_0$  is the shear modulus at zero strain and  $\gamma$  is the nonlinear term. Gokhale *et al* (2008) provide a detailed understanding of the Veronda–Westman model. Substituting (9) into (7) and using the strain invariants, we can relate the axial component of the Cauchy stress to the overall strain as

$$\sigma = 2\lambda^2 \mu_0 e^{\gamma(\lambda^2 - 2\lambda^{-1} - 3)} - \frac{2}{\lambda} \mu_0 e^{\gamma(\lambda^2 - 2\lambda^{-1} - 3)} + \frac{\mu_0}{\lambda^2} - \mu_0 \lambda. \quad (10)$$

Young's modulus is given by  $E = \partial\sigma/\partial\varepsilon$ ; therefore,  $E$  can be written in terms of  $\lambda$ :

$$E = 2\mu_0 \left\{ e^{\gamma(\lambda^2 - 2\lambda^{-1} - 3)} [2\lambda + \lambda^{-2} + 2\gamma(\lambda^3 - 2 + \lambda^{-3})] - \frac{1}{2} - \lambda^{-3} \right\}. \quad (11)$$

Note that  $\mu_0 = E_0/3$ , where  $E_0$  is Young's modulus in the limit as the strain goes to zero, and Poisson's ratio  $\approx 0.5$  corresponding to incompressibility.

## 2.2. Percolation theory

De Gennes (1976) suggested that percolation theory can describe rheology of the solution–gel transition. The gelation process changes the viscoelastic characteristics of the material. The shear modulus after the transition can be denoted as a function of the intermolecular crosslinking probability  $p$ :

$$\mu \propto (p - p_c)^t, \quad (12)$$

where  $p_c$  is the percolation threshold (which characterize the transition from solution to gel) and  $t$  is the critical exponent. In this work the agar concentration was varied. For agar, it is known that the concentration of crosslinks at a fixed temperature is proportional to the concentration of double helix fibrils and that is assumed to be proportional to the agar dry-weight concentration (Hall *et al* 1997, Fujii *et al* 2000).

The percolation theory of gelation implies that the value of  $t$  in equation (12) lies in the range 1.8–2.0 for all gel materials (Ikeda *et al* 1989, Djabourov *et al* 1988). Some authors found critical exponents for the gelatin gel to be in good agreement with percolation theory (Ikeda *et al* 1989, Djabourov *et al* 1988). Fujii *et al* (2000) similarly applied percolation theory to evaluate the elastic properties of the agar gel as a function of its dry weight concentration.

The fact that percolation theory predicts that the shear modulus increases proportionally to the dry-weight concentration following a power law behavior suggests that the shear modulus dependence on the dry weight percentage concentration is truly independent of the compression applied to the gel. From that it follows that gels have the same nonlinearity independent of the material dry-weight concentration.

## 2.3. Materials production

Four types of materials were produced, namely, agar-based material, gelatin-based material, (agar + gelatin)-based materials and oil dispersions in agar + gelatin.

**2.3.1. Agar-based material**—A molten agar solution was obtained by mixing 2 g granulated agar (Fisher Scientific, Pittsburgh, PA, cat. no BP1423) into 100 mL 18 MΩ cm deionized water at room temperature and heating in a ‘double boiler’ to 90 °C until transparency existed. The molten agar was then cooled to 50 °C by immersing the beaker in 18 °C water with stirring. Liquid Germall-plus® (International Specialty products, Wayne, NJ, USA) was added (1.5 g per 100 mL solution) to prevent fungal and bacterial contamination. The agar dry weight concentration of this plain agar sample was 1.93%.

**2.3.2. Gelatin-based material**—Gelatin solutions were obtained by mixing 8 g of 200 Bloom gelatin (derived from calfskin; Vyse Gelatin Company, Schiller Park, IL) per 100 mL of 18 MΩ cm deionized water (7.4% by weight gelatin) at room temperature and heating in a ‘double boiler’ to 90 °C until transparency existed. The molten gelatin was then cooled to 50°C and 1.5 g of the preservative liquid Germall-plus® was added per 100 mL of solution. Then the molten gelatin was further cooled to 36 °C and the formalin solution which is 37% formaldehyde was added such that 0.047 g was present for each gram of (dry weight) gelatin. Formalin was used for cross linking the gelatin which increases the gelatin’s melting point and the elastic modulus (Hall *et al* 1997). The solutions were poured into small molds to congeal for 3 or 4 days. The resulting samples were cylinders 2.6 cm diameter and 1.0 cm thick. One sample of plain gelatin was manufactured containing 7.3% dry-weight gelatin.

**2.3.3. (Agar + gelatin)-based material**—To obtain the agar/gelatin mixtures, molten agar/water solution and molten gelatin/water solution (7.4% by weight gelatin) were mixed together, each at a temperature of about 90 °C, in a proportion of 40% by volume of gelatin solution and 60% by volume of agar-plus-water solution. The mixture was cooled to 50 °C and 1.5 g of liquid Germall-plus® was added per 100 mL of mixture. The mixture was further cooled to 36 °C and 0.047 g formalin added for each dry weight gram of gelatin. The mixture was then poured into cylindrical molds to cool and congeal for 3 or 4 days. A more detailed description of the procedure can be found in Madsen *et al* (2005).

The gelatin/water solution used was always 7.4% dry weight. The agar solution was made with different dry weight percentages from 1 to 4.8%. The dry weight percentage gelatin in the mixture was 3.0% and the dry weight percentages of agar were 0.6% to 2.8%. The final concentrations—accounting for the added formalin and liquid Germall-plus®—are shown in table 1.

It is important to note that the gelatin concentrations are the same for all samples so that if a phantom is to be manufactured with an inclusion, the inclusion and background of phantoms will have the same dry weight concentrations of gelatin to minimize osmotic pressure differences. Equal gelatin concentration in the inclusion and background also helps to bond the inclusions to the background (Madsen *et al* 2003, 2005).

In order to manufacture the fourth type of material (oil dispersions), plain gelatin (7.4%) was poured into plain agar (0.99, 1.96, 2.91 and 3.85% by weight), as shown in table 2. The gel was poured into safflower oil and gently stirred with an appropriate spoon. A surfactant (Ultra Ivory® Proctor and Gamble Company, Cincinnati, OH, USA) was added so that emulsification was sufficient to produce microscopic oil droplets that would not separate from the aqueous gel during either the congealing process or the mechanical test. This test consists of compressing the phantom sample as described in section 2.4. The volume of the surfactant is approximately 1% of the safflower oil volume. The detailed procedure to obtain

the oil in aqueous gel materials was previously described (Madsen *et al* 2005, 2006). The oil volume percentages produced were 0%, 20% and 50%. Therefore, 12 samples were obtained. The oil volume percentages in each of the 12 samples are shown in table 3.

All samples were stored submersed in safflower oil to prevent dehydration.

#### 2.4. Elastic measurements

Mechanical tests were performed on each of the samples to evaluate their stress/strain properties using a Bose EnduraTEC 3200 ELF system (Bose Corporation EletrectroForce Systems Group, Minnetonka, MN, USA) with a 1 kg load cell and Teflon<sup>®</sup> platens larger than the sample surface. Low-amplitude, 1 Hz oscillatory compressive load was used to determine the complex Young's modulus. Large oscillatory deformations (compressions up to 25%) were employed to assess nonlinear characteristics. The loading cycle of the stress/strain curve was chosen for this purpose.

The same oil used to store the materials was also used to lubricate the platens during the tests to minimize friction between the plates and the sample.

After the upper platen established contact with the sample, the platen was lowered to the mean level of oscillation amplitude at a rate of  $0.04 \text{ mm s}^{-1}$ . The platen remained at the mean level for 5 s. In the next step, the sample underwent a sinusoidal compression in the desired amplitude and frequency for 5 s before starting data acquisition. These procedures were done to 'precondition' the sample (Fung 1993, Hall *et al* 1997) and obtain consistent load-displacement measurements. Force and displacements data were then acquired.

Deformations up to 25% were adopted since this amount of deformation should be sufficient to evaluate the nonlinear parameters of tissues being imaged with an ultrasound scanner (Oberai *et al* 2009). Deformation beyond 25% could also have damaged the phantoms, especially those containing agar which is a brittle material. However, no study to verify the fracture point was done.

Considering the use of a 1 kg load cell and the initial diameter of the samples being tested (2.6 cm), the maximum measurable stress in these experiments (not accounting deformation-dependent sample area) would be around 19 kPa.

The results described in this manuscript were obtained from measurements made at least 2 months after the sample production, that is, a reasonable period for the materials to become stable (Hall *et al* 1997, Madsen *et al* 2005, 2006). Two sets of measurements were made for each sample on consecutive days. The results shown below are the average of these two measurements and the errors are the standard deviation (of only two measurements).

### 3. Results

Before evaluating the nonlinear properties of the agar/gelatin and oil-in-agar/gelatin materials, it is useful to first analyze the plain agar (1.93% dry weight) and plain gelatin (7.4% dry weight) materials. The stress versus stretch ratio ( $\lambda$ ) for those materials are shown in figure 1. It is clear that the agar sample stiffens faster than the gelatin (Hall *et al* 1997). The data were fit to the Veronda–Westman constitutive model (equation (10)) using a nonlinear least-squares and the Levenberg–Marquardt methods (Levenberg 1944, Marquardt 1963); resulting values for  $\gamma$  and  $\mu_0$  are shown in table 4. The values of Young's moduli ( $3\mu_0$ ) at zero strain are compared to Young's storage modulus acquired from the oscillatory DMA (dynamic mechanical analysis) at 1% pre-compression and 1% amplitude peak to peak.

The results of the mechanical tests for agar/gelatin samples (table 1) are presented in figure 2. Those data were fit to the Veronda–Westman model and the results are shown in table 5.

From Young's moduli in table 5, the critical exponent  $t$  relating to percolation theory (equation (12)) was  $t = 1.89 \pm 0.02$  in agreement with that previously reported for agar or gelatin materials (Hall *et al* 1997) suggesting that the stiffness of gelatin and agar mixtures for different agar dry weight concentrations obeys percolation theory (that is, the mixtures have the same critical exponent as observed for the plain agar material). The samples presenting a consistent nonlinear behavior (table 5) suggests that the shear modulus dependence on dry weight percentage concentration is truly independent of the compression applied to the gel. That implies that, within this measurement range, the critical exponent would be the same regardless the applied compression to the material (see figure 3).

The stress/strain curves for the oil-in-agar/gelatin materials are represented in figure 4. The graphs were organized so that the samples with the same agar dry weight concentration but different oil volume percentage are shown in the same plot.

The results in figure 4 are well represented by the Veronda–Westmann model ( $R_2 > 0.92$ ). The nonlinear parameter ( $\gamma$ ) obtained from the fits are shown in figure 5. The oil emulsification decreased the nonlinear behavior of the materials. Figure 6 shows the storage moduli obtained from the mechanical tests of the oil-in-agar/gelatin materials. Figure 6(a) represents the actual values obtained. Those values were fit to a power law in order to compare the percolation theory critical exponents (equation (12)) for samples with different oil volume fraction (table 6). Figure 6(b) represents the 20% and 50% oil volume fraction materials' storage moduli relative to the values acquired for the case of 0% of oil. Those plots show that for a higher agar concentration the relative storage modulus reduction is more accentuated than that for lower agar concentrations.

The critical exponent for 0% oil ( $t = 2.3 \pm 0.2$ ; table 6) is in the range of values found in the literature (Fujii *et al* 2000). The fact that the exponents decreased for higher oil volume concentrations agrees with the results of the chart represented in figure 6(b).

## 4. Discussion

These results should enhance our ability to select materials for producing nonlinear elasticity imaging phantoms which mimic clinical situations. For example, knowing the appropriate model functions and how the parameters depend on constituent materials allows predicting stress/strain curves to be used as inputs to nonlinear finite-element simulations. Simulations and development of anthropomorphic phantoms are resulting tools for assessing the validity of promising nonlinear elastographic techniques (Oberai *et al* 2009).

### 4.1. Plain agar and plain gelatin

The nonlinear parameter of the plain agar (1.93% by weight) was ten times greater than that found for the plain gelatin (7.4% by weight; table 4). The fact that the coefficients of determination ( $R^2$ ) for those two extreme cases were quite large indicates that the Veronda–Westmann model is a good model for the nonlinear stress/strain curves of these phantom materials. The shear moduli at no strain (obtained from the Veronda–Westmann model) were compared to the storage moduli calculated by the DMA method. The difference between the results obtained by the two methods was of 7.8% and 4.5% for the plain agar and plain gelatin, respectively, further suggesting that the Veronda–Westmann model is a good choice for these materials and experiments.



## 4.2. Agar/gelatin mixtures

The average nonlinear parameter values ( $\gamma$ ) obtained for the various agar/gelatin mixtures (table 5) was  $4.5 \pm 0.3$ . No trend in  $\gamma$  was observed for different agar concentrations suggesting that the nonlinearity of the agar/gelatin mixtures did not depend on agar concentration in the range from 0.58% to 2.81%.

Young's moduli for the agar/gelatin mixtures obtained from the Veronda–Westmann model were also in agreement with the storage moduli measured via DMA. The critical exponent  $t$  relating to percolation theory was in agreement with that found for plain agar (Hall *et al* 1997). Presenting a consistent critical exponent independent of the applied compression (up to 17%; figure 3) supports the fact that nonlinear behavior remained constant for the range of agar concentration tested. Figure 5 shows that the presence of oil reduced the nonlinear behavior of the samples such that the higher the oil concentration, the lower the nonlinear parameter  $\gamma$ . The  $\gamma$  value for the 20% oil was about 75% of  $\gamma$  for the 0% oil samples, and that for the 50% oil sample was about 45% of the 0% sample.

The decrease in Young's storage moduli with addition of oil was more pronounced at higher concentrations of agar as shown in figure 6. This phenomenon was also observed by Kim *et al* (1999); however, in their case, only plain agar was examined. For both 20% and 50% oil in figure 6 the fractional decrease in stiffness followed a linear behavior with  $Y = -0.084Ag + 0.75$  ( $R_2 = 0.82$ ) for 20% oil and  $Y = -0.105Ag + 0.40$  ( $R_2 = 0.99$ ) for 50% oil where  $Y$  is the ratio of storage modulus with oil to that with no oil and  $Ag$  is the agar concentration (%). Though a power law relationship for stiffness versus the agar concentration still exists, the critical exponent values ( $t$ ) decreased with increasing oil in materials (table 6).

## 5. Conclusion

In this work, the hyperelastic behavior of materials commonly used to manufacture phantoms for elastography was analyzed. Special attention was given to their nonlinear behavior. The stress/strain curves for four sets of samples (plain agar, plain gelatin, agar/gelatin mixtures, oil-in-agar/gelatin mixtures) were fit to the Veronda–Westmann nonlinear model. The elasticity of plain agar was more nonlinear than that of gelatin. The agar/gelatin mixtures showed a nonlinear behavior between that of gelatin and agar. It was observed that adding oil to the agar/gelatin mixtures decreased the stiffness of the gels and also decreased nonlinear behavior. The insights provided by this study could facilitate development of heterogeneous tissue mimicking phantoms for representing mechanical nonlinearities occurring in normal and abnormal tissues.

## Acknowledgments

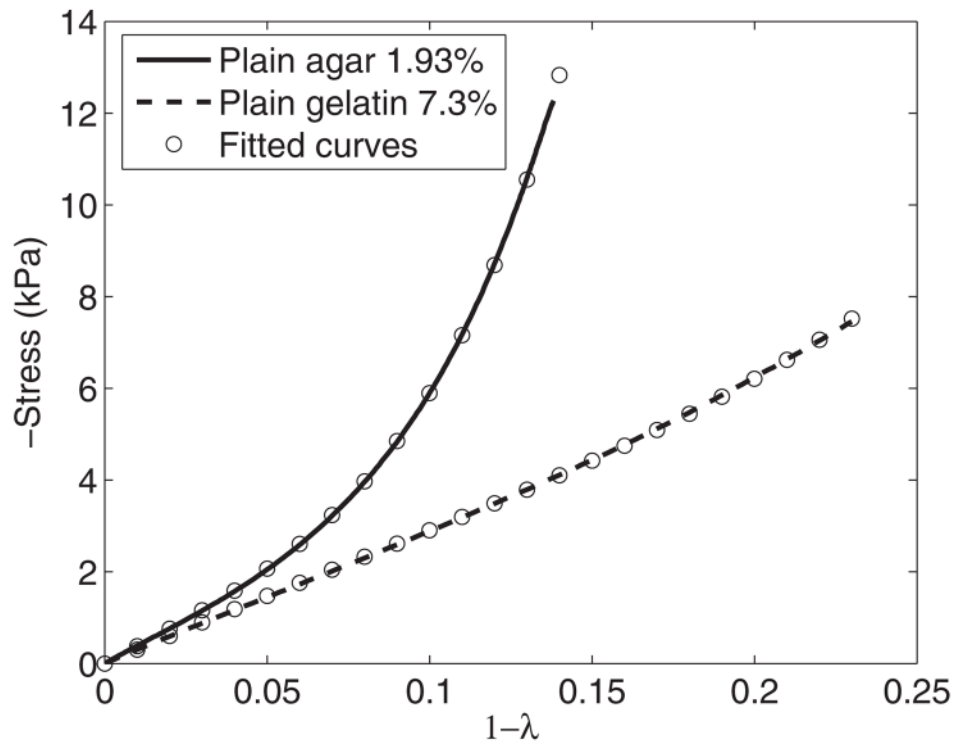
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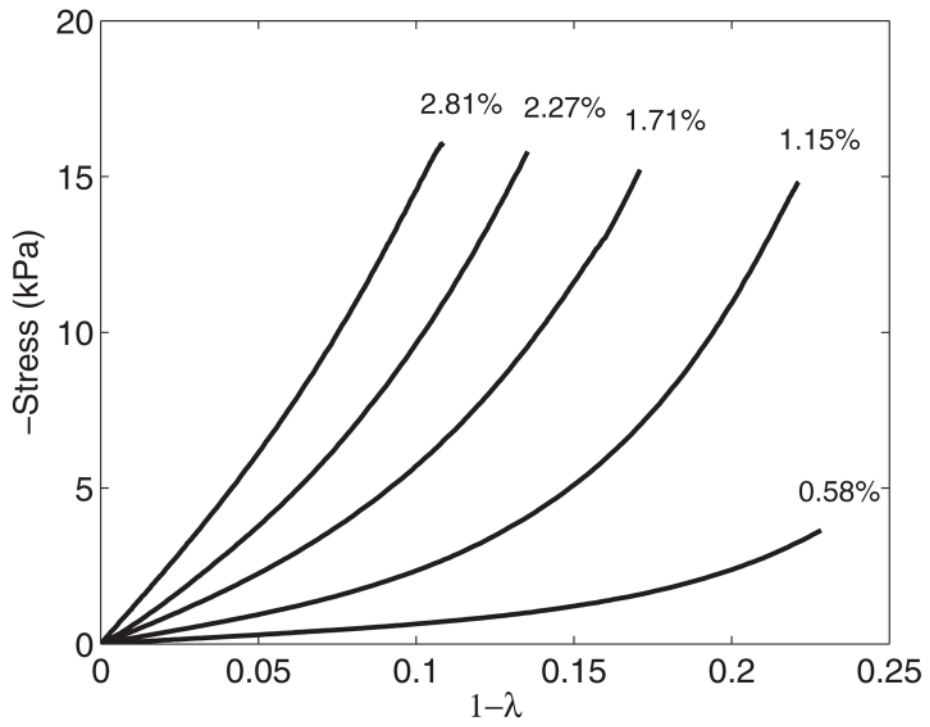
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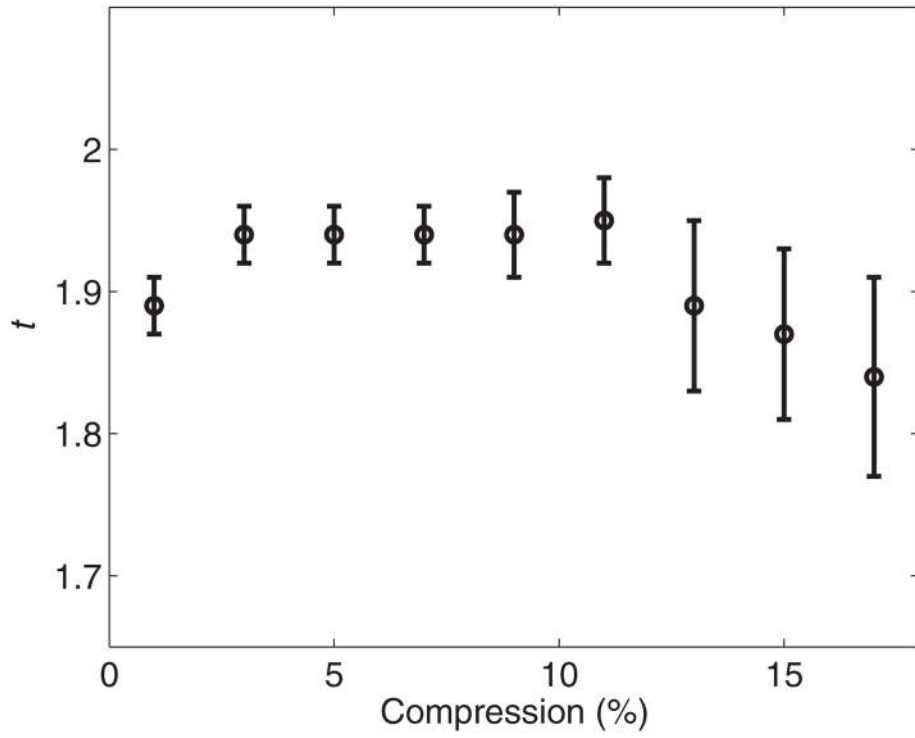
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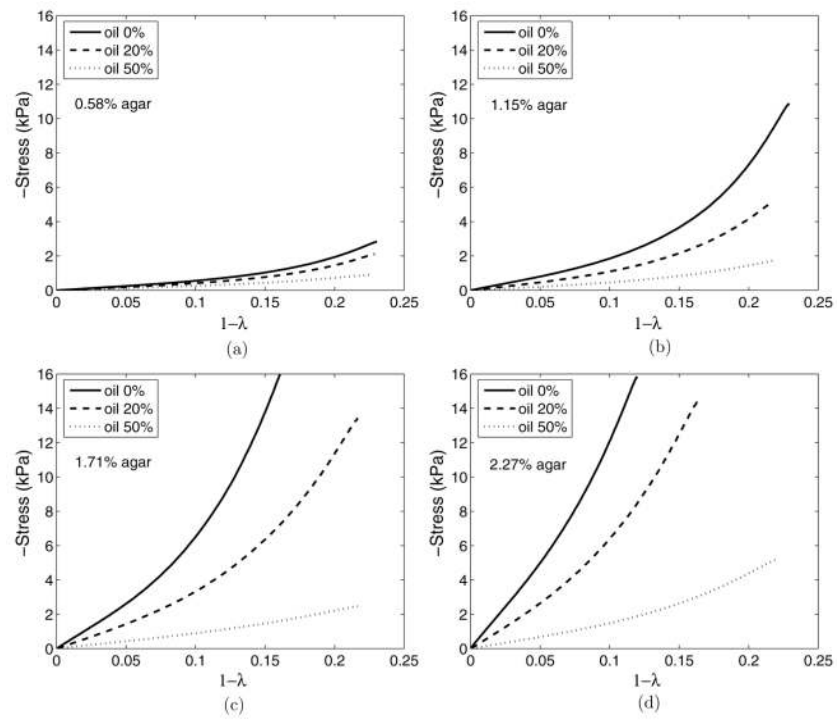
**Figure 1.** Stress (equation (10)) versus stretch ratio ( $\lambda$ ) of the plain agar and plain gelatin materials. The fitted Veronda–Westman model results are also shown for both cases.



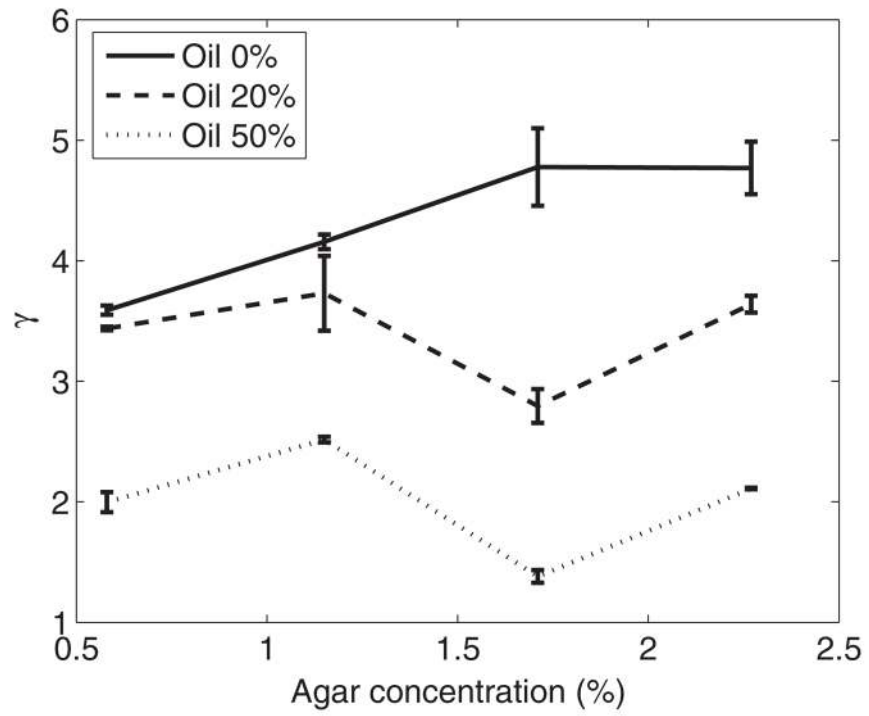
**Figure 2.** Stress versus stretch ratio for the set of agar/gelatin samples with five different dry weight concentrations of agar.



**Figure 3.** Critical exponent ( $t$ ) versus the compression applied to the sample for the (agar + gelatin)-based material.

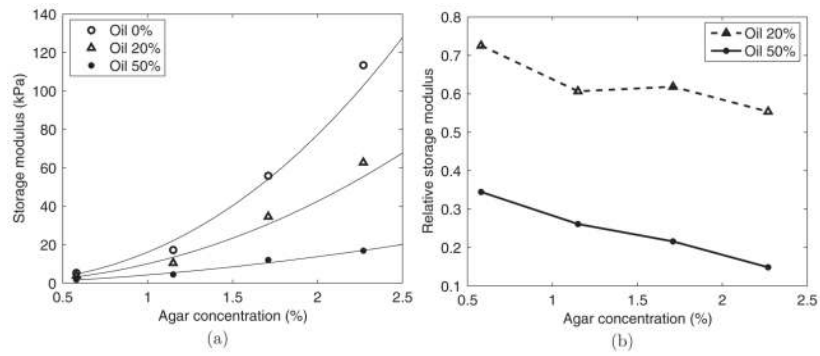


**Figure 4.** Stress versus stretch ratio for the 12 oil-in-agar/gelatin samples with 0%, 20% and 50% oil. The agar concentrations are (a) 0.58%, (b) 1.15%, (c) 1.71%, (d) 2.27%.



**Figure 5.** Nonlinear parameter ( $\gamma$ ) versus agar concentration for the oil-in-agar/gelatin materials.





**Figure 6.**

Young's storage modulus (from DMA calculation) versus agar concentration for the three oil emulsification volumes (1% of compression). (a) Curves corresponding to percolation theory power ( $t$ ) law fits (table 6). (b) Curves representing the percentage storage modulus of the 20% and 50% oil volumes emulsification relative to the 0% oil volume versus the agar concentration.

**Table 1**

Final concentration used to make the agar/gelatin samples.

<b>Sample</b>	<b>Agar (%)</b>	<b>Gelatin (%)</b>	<b>Germall-plus (%)</b>	<b>Formaldehyde (%)</b>
1	0.58	2.94	1.46	0.138
2	1.15	2.94	1.46	0.138
3	1.71	2.93	1.46	0.138
4	2.27	2.93	1.45	0.138
5	2.81	2.92	1.45	0.138

**Table 2**

Materials compounding the gel matrix used to make the oil droplets in agar/gelatin samples.

<b>Sample</b>	<b>Agar (%)</b>	<b>Gelatin (%)</b>	<b>Germall-plus (%)</b>	<b>Formaldehyde (%)</b>
1-3	0.58	2.94	1.46	0.138
4-6	1.15	2.94	1.46	0.138
7-9	1.71	2.93	1.46	0.138
10-12	2.27	2.93	1.45	0.138

**Table 3**

Oil volume percentage for the samples presented in table 2.

Sample	Oil volume (%)
1; 4; 7; 10	0
2; 5; 8; 11	20
3; 6; 9; 12	50

Fitting parameters  $\gamma$  and  $\mu_0$  for the plain gelatin (7.3% dry weight) and plain agar (1.93% dry weight).  $R^2$  stands for the coefficient of determination.

**Table 4**

Sample	$\gamma$	$\mu_0$ (kPa)	Model $E$ (kPa)	DMA $E$ (kPa)	$R^2$
Plain gelatin 7.3%	$0.9 \pm 0.1$	$10.1 \pm 0.2$	$30.2 \pm 0.6$	$28.9 \pm 0.3$	1.00
Plain agar 1.93%	$9.0 \pm 0.2$	$12.7 \pm 0.1$	$38.8 \pm 1.2$	$41.6 \pm 1.9$	0.99

Parameters  $\gamma$  and  $\mu_0$  obtained by fitting the data shown in figure 2 to the Veronda–Westman model (equation (10)) and the corresponding coefficient of determination ( $R^2$ ). A comparison of Young's modulus at zero strain ( $3\mu_0$ ) to results estimated with the EnduraTEC DMA software is also provided.

**Table 5**

Sample	$\gamma$	$\mu_0$ (kPa)	Model $E$ (kPa)	DMA $E$ (kPa)	$R^2$
0.58% agar in gelatin	4.1 ± 0.2	1.8 ± 0.1	5.3 ± 0.1	6.3 ± 0.1	0.93
1.15% agar in gelatin	4.8 ± 0.1	6.7 ± 0.2	20.0 ± 0.7	23.7 ± 0.8	0.95
1.71% agar in gelatin	4.3 ± 0.1	15.8 ± 0.2	47.5 ± 0.4	44.7 ± 3.1	0.92
2.27% agar in gelatin	4.7 ± 0.5	25.0 ± 0.9	79.6 ± 9.3	82.2 ± 7.1	0.84
2.81% agar in gelatin	4.7 ± 0.7	39.3 ± 2.2	117.8 ± 6.7	132.0 ± 3.0	0.89

**Table 6**

Critical exponents obtained for the oil-in-agar/gelatin samples.

Sample	$t$	$R^2$
Oil 0%	$2.3 \pm 0.2$	0.98
Oil 20%	$2.1 \pm 0.2$	0.97
Oil 50%	$1.7 \pm 0.2$	0.98