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Modeling Ramp-hold Indentation Measurements based on Kelvin-Voigt Fractional Derivative Model

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Abstract

Interpretation of experimental data from micro- and nano-scale indentation testing is highly dependent on the constitutive model selected to relate measurements to mechanical properties. The Kelvin-Voigt Fractional Derivative model (KVFD) offers a compact set of viscoelastic features appropriate for characterizing soft biological materials. This paper provides a set of KVFD solutions for converting indentation testing data acquired for different geometries and scales into viscoelastic properties of soft materials. These solutions, which are mostly in closed-form, apply to ramp-hold relaxation, load-unload and ramp-load creep-testing protocols. We report on applications of these model solutions to macro- and nano-indentation testing of hydrogels, gastric cancer cells and ex vivo breast tissue samples using an Atomic Force Microscope (AFM). We also applied KVFD models to clinical ultrasonic breast data using a compression plate as required for elasticity imaging. Together the results show that KVFD models fit a broad range of experimental data with a correlation coefficient typically $R^2 > 0.99$. For hydrogel samples, estimation of KVFD model parameters from test data using spherical indentation versus plate compression as well as ramp relaxation versus load-unload compression all agree within one standard deviation. Results from measurements made using macro- and nano-scale indentation agree in trend. For gastric cell and ex vivo breast tissue measurements, KVFD moduli are, respectively, $1/3 - 1/2$ and $1/6$ of the elasticity modulus found from the Sneddon model. In vivo breast tissue measurements yield model parameters consistent with literature results. The consistency of results found for a broad range of experimental parameters suggest the KVFD model is a reliable tool for exploring intrinsic features of the cell/tissue microenvironments.

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Keywords

KVFD models; ramp-hold indentation; AFM measurements; hydrogels; tissue samples

1. Introduction

The architecture of soft biological tissues may be characterized by features describing its biphasic, viscoelastic response to an applied load. Tissue viscoelasticity varies with collagen type, molecular arrangement, cross-linking density, and properties of interstitial fluids, which are all modified by disease processes involving inflammation.

Viscoelastic tissues possess time-dependent behavior or lag. Dynamic testing often uses harmonically-oscillating load stimuli applied by a compressor to quantify the phase lag at specific frequencies [1–5]. In contrast, indentation applies a focal load using a ramp-hold or a periodic ramp-on-ramp-off stimulus to measure time-dependent behavior of materials at low load frequencies (<1 Hz). The full force-displacement time response is monitored during contact-mechanics testing techniques [6–11].

There are a variety of mechanical testing approaches applied to characterize viscoelasticity of soft tissues. Viscoelasticity is usually characterized non-parametrically by a complex modulus where elasticity is depicted by the storage modulus and viscosity by the loss modulus in frequency domain [4, 5, 12]. Elasticity is also depicted non-parametrically by the stiffness (slope of the stress-strain curve) and by the loss energy (area enclosed by the stress-strain curve) in the time domain [1–4,12][13]. Results from both approaches show an ability to differentiate normal and pathological tissues. For example, benign prostatic hyperplasia was found to be significantly softer in elastic modulus than healthy prostate tissues while prostate cancers were found to be stiffer [2]. Fibroglandular breast tissues exhibit a broad range of strain-dependent shear modulus values [2]. Samani et al. reports that many breast cancers exhibit substantially higher Young's modulus values than fibroglandular tissues, while fibrocystic lesions present with a moderately elevated modulus [1]. Fresh-frozen cadaveric feet tissue obtained from diabetic vs. non-diabetic patients demonstrated altered mechanical properties with significantly increased stiffness but no change in energy loss [3]. The storage modulus of tumor-to-background contrasts was much lower in primary than in secondary human hepatic cancers [4]. Ablated regions in *ex vivo* bovine liver tissue showed low stiffness contrast on the periphery of the thermal lesion but high stiffness in the central zone [5,13]. The modulus of the uterine tissue contrast by ramp testing was comparable to that of dynamic testing at low strain rate [12]. Uterine leiomyomas are shown to be stiffer than normal tissue and the single carcinoma specimen was softer than normal tissue [12].

Viscoelasticity has also been quantified parametrically using a mechanical model involving spring and dash pot elements to describe elastic and viscous properties. Viscoelastic properties are widely summarized by the elastic modulus, fluidity and retardation times when time-dependent response curves (indentation testing) [10,11][14–18] [19] or frequency-dependent complex modulus (dynamic or indentation testing) [16,18][20, 21] were fit to a representative model. Parametric approaches offer the advantage of describing structural changes in tissues relate to disease processes. The canine prostate urethra shows

up as a soft region (lower modulus) in the modulus image, and the inner medulla of ovine kidney shows up as a soft region while the outer cortex a stiffer region [19]. The stiffness of the scaffold characterized through atomic force microscopy (AFM) is found to increase significantly due to the extracellular matrix deposited from cells that are grown within the scaffold [11]. Elastic moduli and local retardation times were extracted from creep data when a second-order Kelvin-Voigt model was applied [10]. Similar work was proposed in [16] that used a fractional Kelvin-Voigt model to fit the data. The latter results show that viscosity parameters such as fluidity or retardation time can be used to differentiate normal and cancerous tissues.

Indentation methods offer the possibility of estimating surface properties in heterogeneous media with high spatial resolution by varying the shape and size of the probe. Indentation testing can be adapted to a broad range of spatiotemporal scales. It has been adapted to soft materials such as polymers, hydrogels, emulsions, and glassy materials, cells and tissues [6] [22–24]. Recent results using nano-indentation to evaluate hydrated materials and soft tissues are reviewed in [6][25, 26].

Indentation techniques are based on contact theory [27]. They provide models for arbitrary tip geometries assuming an elastic material in an infinite half plane [7,22,25][28]. The most widely used are Hertzian for a spherical indenter and Sneddon for conical-pyramidal indenters [23]. Thanks to development of the Boltzmann integral superposition principle [29, 30], viscoelastic behavior of soft media with an arbitrary shape indenter by quasi-static stimuli is possible by first selecting a model that accounts for the measurement geometry such that force-displacement measurements can be converted into intrinsic material properties. Since most biological materials exhibit complex viscoelastic behavior, classical elementary models such as Maxwell, Kelvin–Voigt and standard linear solid Zener cannot accurately describe the complex viscoelastic responses of biological materials with arbitrary shape [31–33]. Cascaded elementary models can improve model fits but lead to overfitting with noisy measurements. They also increase the number of model parameters, which complicates interpretation of parametric results and increases computational costs [34, 35]. Another problem with classic integral models is their creep compliance and relaxation modulus are expressed as a Prony series that sometimes has no closed-form analytic solution for ramp-hold indentation tests [34]. One resorts to Prony-form models for force-displacement data only during the holding phase of a ramp-and-hold stimulus, although it is difficult to interpret these model parameters in biphasic media like biological tissues. (Biological cells composing living tissues can change their internal structure, especially during disease processes, to modify their individual properties and those of the tissues they compose [36]). Describing viscoelastic behavior of biological tissues poses unique challenges when compared with inert materials. Up to now, AFM data were analyzed using primarily elastic models fit only to a portion of the indentation response to avoid the viscous responses.

Fractional-derivative models are a combination of spring and fractional-order dashpot elements that reflect both elastic and time-dependent viscous mechanical behavior in a natural way. They first gained prominence when it was shown they can fit polymer experimental data well with few model parameters [29][37]. These mathematical

expressions have been adapted to a variety kind of viscoelastic mechanical response. In [38, 39], Fractional derivative model was applied to three areas of bioengineering research (bioelectrodes, biomechanics, bioimaging) to build new mathematical models. Fractional-order models, such as fractional Kelvin-Voigt, fractional Maxwell, and fractional Zener, are now being applied to a wide range of material assessment problems in bioengineering because they show the flexibility needed to model soft materials. They have also been applied to response data recorded from liver, prostate and breast tissues [7,16,20,21,37,38] [40–42]. However, there has been much confusion in reported literature values because the experimental details are not the same between labs [9]. The influences of experimental conditions such as indenter shape and size on the measurements are widely reported [6–9,34] [43]. Results from different indentation tests can vary because fitting only a portion of the time series remains sensitive to the starting and ending points [44], as well as the choice of model. The consistency of material properties found between tests with varying geometries provides confidence that measurement-independent properties have been found.

Another problem arising in indentation testing is measurement variability from different loading methods. Step loading is preferred for stress relaxation and creep testing for analytical convenience. However, it is difficult to apply a near-step strain without creating oscillating transients that are more representative of the measurement device than the material. In contrast, ramp-hold loading strategy can dramatically improve experimental data stability and improve the reliability of model fitting but only if the ramp-hold stage is included in the model [14,34, 43][45, 46].

Our overall research goal is to seek a consistent viscoelastic model from one theoretical framework that applies to a variety of ramp-hold indentation stimuli. Among fractional derivative models, KVFD has shown an ability to represent time-dependent mechanical behavior for several biological materials and tissues. It offers a good balance between model simplicity and experimental fitting accuracy [14,15,31].

Previous results have shown that KVFD model parameters can differentiate normal from cancerous tissues [16]. Also, dynamic testing of *in vitro* canine liver samples showed an ability to detect thermal lesions consistent with ablation techniques [21]. In that study, the KVFD model shows closer agreement among samples at frequencies less than 100 Hz than a standard Kelvin-Voigt (KV) model. Elastic contrast between cancerous and normal prostate tissues was quantified to show it increases with stimulus frequency [17,20]. KVFD is also proved suitable for describing the viscoelastic properties of porcine liver in both time and frequency domains [18].

In our previous work, we derived the KVFD model in closed form for the ramp-hold relaxation experiment, and showed it can accurately represent the entire experimental data record. We also found that KVFD model parameters can be interpreted in terms of tissue composition and structure [14].

In this paper, we extend our modeling work to include more general ramp-hold indentation testing based on the KVFD model. First, analytical solutions to different ramp-hold indentation tests using different indenter shapes (spherical, conical-pyramidal and plate) are

derived by utilizing basic indentation theory and Boltzmann hereditary integral operators for viscoelastic materials. Model fitting is found to be more reliable when the ramp-hold phase of the response is included. Second, different indentation tests are simulated and several influencing factors are discussed from the parametric modeling point of view. Finally, these models are applied to indentation tests performed to estimate viscoelastic properties of soft materials like hydrogels, cells and tissues. The adaptability to KVFD models to different indentation test from macro- to nano-scales is explored.

2. Methods

2.1 KVFD model

The KVFD model provides an accurate and flexible method for describing material properties with only three parameters [14,15,31]. The constitutive equation relating stress $\sigma(t)$ to strain $\varepsilon(t)$ for the fractional-derivative Kelvin-Voigt model is expressed as [30,35]

$$\sigma(t) = E_0 \varepsilon(t) + \eta \frac{d^\alpha \varepsilon(t)}{dt^\alpha} \quad (1)$$

Where $\eta = E_0 \tau^\alpha (\text{Pa} \times \text{s}^\alpha)$. E_0 is an elastic modulus (Pa), η is a viscous coefficient with time constant $\tau(s)$, and α is a unitless real number between (0,1) that defines the derivative order.

2.2 Basic viscoelastic quantities

The Boltzmann hereditary integral operators for viscoelastic materials state [29,30]

$$\sigma(t) = \int_{-\infty}^t G(t-\tau) \frac{d\varepsilon(\tau)}{d\tau} d\tau \quad (2-1)$$

$$\varepsilon(t) = \int_{-\infty}^t J(t-\tau) \frac{d\sigma(\tau)}{d\tau} d\tau \quad (2-2)$$

where $G(t)$ is the relaxation modulus and $J(t)$ is the creep compliance. Both are time-dependent functions. Since viscoelastic materials have memory, the current state of stress/strain will depend on past deformations.

The Laplace transforms of Equations (1) and (2) give

$$\sigma(s) = E_0 \varepsilon(s) + E_0 \tau^\alpha s^\alpha \varepsilon(s) = s G(s) \varepsilon(s) \quad (3)$$

$$\text{and } \varepsilon(s) = \frac{1}{(E_0 + E_0(s\tau)^\alpha)} \sigma(s) = sJ(s)\sigma(s). \quad (4)$$

From Eqs. (3) and (4), we obtain

$$G(s) = (E_0 + E_0(s\tau)^\alpha)/s \quad (5-1)$$

$$\text{and } J(s) = \frac{1}{s(E_0 + E_0(s\tau)^\alpha)}. \quad (5-2)$$

Applying the inverse Laplace transform yields the relaxation modulus for the KVFD model

$$G(t) = E_0 + \eta \frac{t^{-\alpha}}{\Gamma(1-\alpha)} = E_0 \left[1 + \frac{(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right] \quad (6-1)$$

where $\Gamma(\cdot)$ is a gamma function.

Similarly, creep compliance is given by

$$J(t) = \frac{1}{E_0} \left(\frac{t}{\tau} \right)^\alpha \mathbf{E}_{\alpha, 1+\alpha} \left(-\left(\frac{t}{\tau} \right)^\alpha \right) = \frac{1}{E_0} \left[1 - \mathbf{E}_\alpha \left(-\left(\frac{t}{\tau} \right)^\alpha \right) \right] \quad (6-2)$$

where $\mathbf{E}_{\alpha, \beta}(z) = \sum_{k=0}^{\infty} \frac{z^k}{\Gamma(\alpha k + \beta)}$ is the Mittag-Leffler (M-L) function. For $\beta=1$, it is written as the M-L function in one parameter, i.e., $\mathbf{E}_{\alpha, 1}(z) \equiv \mathbf{E}_\alpha(z)$.

2.3 Solutions to ramp-hold relaxation and load-unload hysteresis tests

The time-series response recorded by the indenter is a load-displacement curve with a stress-relaxation or creep-recovery phase where the maximum displacement or load is held constant. These are the raw data used to estimate stress-strain behavior and ultimately the viscoelastic properties.

According to the generalized Boltzmann integral expression of the stress-strain relationship, the response for indentation in the force-displacement form is given by [34]:

$$\begin{cases} P(t) = K_G \int_0^t G(t-\tau) \frac{dh^n(\tau)}{d\tau} d\tau \\ h^n(t) = \frac{1}{K_G} \int_0^t J(t-\tau) \frac{dP(\tau)}{d\tau} d\tau \end{cases} \quad (7-1) \quad (7-2)$$

where $h(t)$ is the indentation depth, $P(t)$ is the applied force, and γ is Poisson's ratio ($\gamma=0.5$ for incompressible media). K_G is the indenter geometry term given by $K_G = \frac{8\sqrt{R}}{3}$, $\pi/(2 \tan(\theta))$ and $1/(2 \tan(\theta))$ for the spherical, conical, and pyramidal indenters, respectively, where R is the radius of a spherical probe and θ is the half angle of a conical-pyramidal probe. Finally, n is an indentation constant, such that $n = 3/2$, 2, and 1 for spherical, conical-pyramidal, and plate indenters, respectively.

The ramp-hold controlled displacement and force functions are given by

$$h(t) = \begin{cases} vt, & 0 \leq t \leq T_r \\ h_0 = vT_r, & t \geq T_r \end{cases} \quad (8-1)$$

$$P(t) = \begin{cases} vt, & 0 \leq t \leq T_r \\ P_0 = vT_r, & t \geq T_r \end{cases} \quad (8-2)$$

where T_r is the duration of the ramp and v is the velocity of the indenter tip or the rate at which force increases during the ramp-on time.

The displacement function in the load-unload hysteresis test is given by

$$h(t) = \begin{cases} vt, & 0 \leq t \leq T_r \\ v(2T_r - t), & T_r \leq t \leq 2T_r \end{cases} \quad (8-3)$$

Combining Eqs. (7) and (8), solutions to the ramp relaxation/creep and load-unload response can be derived. The detailed derivation is provided in Appendices I–III. Tables 1–3 display the solutions to different indentation tests by a spherical indenter, a conical-pyramidal indenter and a plate indenter.

3. Simulations

To study the influences of experimental factors on the indentation response and to investigate the physical interpretation of model parameters, numerical implementation of the indentation response under different experimental factors (indenter diameter, indentation

depth/peak force, loading rate (ramp time)), and model parameters (E_0 , α , τ and T_r) are conducted. As the indentation response from different indenter shapes is similar, spherical indentation is chosen as representative. In simulation, the parameter ranges span those of the experimental testing configurations for tissues and gelatin-cream emulsion samples [14]. Default testing parameters are $h_0 = 1$ mm, diameter $D = 5$ mm, $P_0 = 1$ N and ramp time $T_r = 25$ s. Default model parameters are $E_0 = 2830$ Pa, $\alpha = 0.163$ and $\tau = 8.31$ s.

a) Indentation response under different experimental factors—The ramp-hold relaxation and load-unload responses predicted by Eqs. (9) and (10) are shown in figure 2 a1–a2. The indentation depth h_0 ranges between 1–2.5 mm at 0.5 mm increments. Larger values of h_0 generate larger values of P_0 . The ramp-load creep response predicted by Eq. (11) is shown in figure 2 a3. Peak force P_0 is set to 1 N, 1.5 N, and 2 N as indicated.

The influence of indenter diameter D on ramp-hold relaxation, load-unload and ramp-load creep responses are displayed, respectively, in figure 2 b1–b3. Larger values of D result in larger force responses but lower displacement responses.

As shown in figure 2 c1–c3, T_r has little influence on the steady-state force response and the force/displacement response, but will influence the shape of the ramp-hold curve.

For conical-pyramidal indentation, the influences of half angle θ is similar to that of the indenter diameter D in that larger values of θ result in larger force responses and lower displacement responses as shown in figure 2 d1–d3.

b) Indentation response under different model parameters—The ramp-relaxation and load-unload responses are displayed in Fig 3 for different model parameters (E_0 , α and τ). The range of modulus E_0 is from 100 Pa to 3100 Pa, fractional order α is from 0.1 to 0.7 and time constant τ is from 2 s to 2000 s.

The elastic modulus parameter E_0 describes material stiffness. Samples with larger E_0 values have more resistance to deformation, thus requiring a larger applied compressive load to achieve a preset displacement value. As shown in figure 3(a1–a3), E_0 determines the height of the force/displacement response.

Fractional-derivative-order parameter α describes the fluidity of a biphasic viscoelastic material. Small α values correspond to higher coefficients of consolidation, which limits the rate at which a material can be compressed. Samples with large α values are more permeable to interstitial fluid flows. They require smaller external forces and less compression time to achieve a preset displacement. Large α values generate a small force peak and large displacement peak; the applied compressive energy dissipates quickly as force/displacement return to equilibrium values.

Recovery-time parameter τ describes the time required for the loaded material to reach an equilibrium state. τ is highly correlated with viscosity whereby larger values recover more slowly. Viscous materials often exhibit significant internal friction that requires larger external force to achieve a preset displacement. As shown in figure 3 (c1–c2), materials with large τ have higher viscosity, which results in higher force peak responses and slower

displacement responses. Finally, as stated in Section 2.1, $\eta = E_0^{\tau\alpha}$ which shows how the three KVFD parameters can be combined to define viscosity.

4. Applications

The aim of this section is to verify that the KVFD model adapts to different indentation tests to give similar parameter estimates for hydrogel, cell, and tissue samples. The tests span a range of indenter sizes, from AFM-type force mapping to clinical ultrasound breast imaging data. Experimental data are fit to model equations using the minimum least-squares fitting criterion.

Figure 4 illustrates the basic indentation testing protocols in the following experiments, where the loading method (inset) and the indentation response curves are displayed.

4.1 Micro-indentation tests on gel-cream samples

Hydrogel samples are made from a combination of gelatin powder, deionized water, and a commercial skin cream, each component in precise weight concentrations as described in [15]. Samples with 5% gelatin and varied cream concentrations (5%, 20%, 30%, and 50% by weight) were made. Variable cream concentration will change the viscosity of the samples. Samples labeled G5C5 contain 5 g gelatin, 5 g Vanicream, and 90 g deionized water per 100 g of sample material. At each concentration, six samples were tested three days after they were constructed once gelatin cross linking has stabilized. Mechanical tests were conducted using the TA-XT plus Texture Analyzer (Texture Technologies, Algonquin, IL USA) with a 5-mm-diameter spherical stainless-steel indentation probe and plate indenter. Ramp-hold relaxation and load-unload hysteresis test tests were conducted on samples.

a) Indentation tests by different loading schemes—Samples were tested using a spherical indenter while bonded to cylindrical molds. The rigid molds used to form samples for spherical indentation testing are 50 mm in diameter and 20 mm in height. Sample sizes were shown to be large enough to avoid significant boundary influences [14].

The samples were subjected to ramp-relaxation and load-unload hysteresis testing. The maximum indentation depth was 1mm and the ramp speed (probe velocity) was $v = 0.04$ mm/s delivered during a ramp time of $T_r = 25$ s.

Representative KVFD fitting of the measurement data is provided in figure 5. The predicted force response (dot-dashed lines) of ramp relaxation as expressed by Eq. (9) is shown along with test data (different markers). The predicted force response (lines) for load-unload testing as expressed by Eq. (10) is displayed with measurement data (different markers). The fitting of ramp relaxation data to the model is precise with $R^2 > 0.9963$. The fitting of the load-unload curve to the model is also precise with $R^2 > 0.9683$. The estimated parameters from 2 different load schemes are provided in Table 4. It can be seen clearly that KVFD model parameters estimated from two load schemes are close and they agree within one standard deviation. The results indicate the adaptability of KVFD to different loading schemes.

b) Indentation tests by different indenter shapes [15]

Ramp-relaxation indentation testing was conducted with two probes: a spherical-tipped indenter with 5mm diameter and a plate compressor having a diameter larger than the cylindrical samples with enough lubrication to approximate a free-slip plate boundary. The emulsion samples were made with 5% gelatin and variable cream concentrations of 5, 15, and 50%. Samples tested using the spherical indenter were bonded to their cylindrical molds, while samples tested using a plate compressor were removed from the mold and placed on a flat immovable surface with its side boundaries free to expand. Samples tested using plate compression were 37.5 mm in diameter and 19 mm in height.

Samples were held for 300 s to measure stress relaxation. Representative KVFD model fits for samples with different cream concentrations are provided in figure 6A (a) – (b).

Predicted responses as expressed in Eq. 9 were fit to spherical ramp relaxation data, and predicted responses expressed in Eq. 15 were fit to plate ramp relaxation data. Given that $R^2 > 0.996$, the results suggest that KVFD models can accurately describe the viscoelastic responses of hydrogel samples from different indenter geometries. KVFD parameters obtained from spherical-tip indentation and plate-compression geometries, as illustrated in figure 6B (a)–(c), agree within one standard deviation [15].

4.2 Nano-indentation testing of gel-cream samples

The gel-cream samples with 5% gelatin concentrations and 4 different cream concentrations (5%, 20%, 30%, and 50% by weight) were made as described in [15]. Three batches of samples at three days are tested using Piuma Chiaro nanoindentation. The loading scheme is approach-hold-retraction, and the ramp-hold stage was analyzed. The radius of the nano-spherical probe is 45.5 μm , and force constant of the probe is 0.5 N/m. The ramp-on time is determined by the preset indentation depth and the approaching speed of the tip. The force can be calculated by multiplication of force constant and the deflection of the tip that was measured by the interferometer. Every sample is automatically tested over a region sampled with a 6×6 matrix of points. KVFD models (Eq. 9) fit representative experimental data well ($R^2 > 0.993$) as shown in Figure 7.

Figure 8 (a)–(c) displays the viscoelastic parameters (E_0 , α and τ) versus cream percentage. Modulus E_0 is maximum near 20% cream percentage, α clearly increases with cream percentage, while τ also increases but the trend is less clear. The values measured from macro-indentation and nanoindentation tests have some differences due to the fact that the geometry of indentation at different scales can activate different material properties, which influence model-parameter estimates. In addition, the ramp time for every sample is varied in nanoindentation testing due to variations in the starting contact point measurement, which may also influence model fitting as we showed previously [14]. Results that are consistent over scale and indentation geometry further strength the utility of the KVFD model.

For gel-cream samples, KVFD model parameters from indentation testing under spherical indenter versus plate compression and different loading scheme (ramp relaxation versus load-unload) are consistent within one standard deviation. The results from different scales

(macro- versus nano-) have a consistent trend although the values are different. These indentation test yield consistent estimates of KVFD model parameters.

4.3 Fitting the force map of gastric cells by AFM with a pyramidal tip

Mechanical properties of gastric cancer cells were tested by Keysight5500 atomic force microscopy in contact mode [47]. The loading scheme is approach and retraction (load-unload). For AFM-type indentation, the retraction process starts when the voltage exceeds the preset value. The indentation depth and ramp time measurements were initiated at the contact point. The pyramidal-conical tip (PointProbe Plus PPP) was used with a force constant of 0.2 N/m, and the sensitivity is calibrated to be 53.536 nm/V at room temperature. The tip approaching speed is 2.93 $\mu\text{m/s}$ and preset voltage threshold is 5 V. The force can be calculated by the multiplication of the force constant and the deflection of the tip that was measured by the four quadrant sensor, where the deflection is the multiplication of the voltage (V) change and probe sensitivity (m/V).

Gastric cancer cells were cultured in 1640 nutrient solution. 10 μM apigenin (APG, type A106675-20 mg) were added to the adherent cells and tested in 24 hours. Apigenin has demonstrated effectiveness against a wide range of cancer types, while not showing toxicity to normal cells [48]. APG are expected to inhibit the cancer cell growth and induce apoptosis that may collapse the skeleton of the cell. Measurements of cells from APG groups are compared with that of control groups (without adding APG). Before testing, optimum solution is used to flush the floating cells. For each sample, 30 cells are tested. A cell is examined by placing the indenter tip directly on top. KVFD fitting of representative experimental data were shown in time versus force as shown in figure 9 (a1–a2). To compare, figure 9 (b1–b2) displays the same displacement versus force data during the loading stage as it is fit to the widely-used Sneddon model. This model gives the force-displacement relationship as

$$f = \frac{2E}{\pi(1 - \nu^2)} \tan(\alpha) \delta^2. \quad (18)$$

The KVFD model of Eq. (13) closely predicts the load-unload force data ($R^2 > 0.99$) for these gastric cancer cells. However, the Sneddon model fits only the approaching stage of the displacement-force curve and with lower R^2 value as it assumes elastic media. Tables 5 and 6 give the best-fit parameters for the KVFD and Sneddon models, respectively. The elastic modulus E_0 in the KVFD model is 1/3 - 1/2 of that for the Sneddon model.

4.4 Fitting the force map of breast tissues via AFM with pyramidal tip

This study was approved by the Institutional Review Board/the Ethics Committee of the first affiliated hospital of Xi'an JiaoTong University (permit number: 2013-0726). Oral informed consent was obtained from 2 participants.

Fresh breast tissue obtained from breast puncture that was fast frozen in liquid nitrogen (-196°C) was transported to the AFM testing laboratory. Frozen tissue slices 60 μm thick

were then made by a freezing microtome. For each tissue sample, three slices were obtained for analysis. Testing time for each slice is about 10 minutes at room temperature. It was found that elastic properties measured in refrigerated fresh tissues up to two hours [1,2] and up to 72 hours [49] post excision did not change. Others measured minimal changes in fresh versus frozen connective tissue samples [3, 13]. However, differences between benign and malignant breast lesion tissues have not been studied.

The tissue slices were tested by Keysight5500 atomic force microscopy in contact mode. Before testing, a drop of physiological saline was placed on the surface of the tissue to maintain the osmotic pressure and position the probe into the solution. Tests were conducted with pyramidal-conical Tip (PointProbe Plus(PPP)). The loading scheme is approach and retraction (load-unload), and the force constant is 0.2 N/m. The deflection sensitivity was calibrated as 50.1 nm/V in the experiment. The tip approaching speed is 2.93 $\mu\text{m/s}$.

A total of 10 measurement points per slice were obtained. The experimental data was fitted to the KVFD theoretical solution as expressed in equation (13). Figure 10 (a1–a2) shows the best fit of the KVFD model to the benign and malignant tissues, respectively, where the correlation coefficients for the fits are $R^2 > 0.99$. In comparison, figure 10 (b1–b2) shows, respectively, the Sneddon fitting (Eq.18) of benign and malignant tissues during the loading stage of the displacement-force curve. The fits consistently resulted in $R^2 < 0.98$ as we tried to fit this elastic model to data from viscoelastic media. Table 7 and table 8 give the best-fit parameters by KVFD model and Sneddon model. The elastic modulus E_0 in the KVFD model is about 1/6 of that of the Sneddon model. The larger α value in the malignant lesion tissue indicates the cell membranes have higher fluidity.

4.5 Fitting creep curves from clinical breast data

Clinical breast ultrasound data was obtained from the UIUC website <http://ultrasonics.bioengineering.illinois.edu/project5.asp>. The data were acquired using a Siemens Sonoline Antares ultrasound scanner (Siemens Medical Solutions USA, Inc., Mountain View, CA) with a VF10-5 linear array transducer operated at 8 MHz. The temporal sampling frequency of the Antares system is 40 MHz. Force was measured by force sensors (ATI Industrial Automation, Apex, NC) mounted to the transducer and a ramp force was applied to the patients [16] [50, 51]. The net force applied was $4.0 \text{ N} \pm 0.24 \text{ N}$ and the ramp time T_r was 0.8 s. Patients were positioned supine and the breasts were scanned anterior-posterior with the chest wall as compression support [50][52]. During acquisition time, patients were asked to hold their breath to minimize respiration-induced strain artifacts [50]. RF frames were recorded for 12 to 15 s and RF acquisition frame rate was 17 fps. The average imaging depth is 30 mm.

These RF data were processed beginning with displacement estimation [53] followed by strain estimation via Kalman filtering[54]. The window size was 125 points (approximately 1.767mm in depth). Strain images are formed by down sampling the displacement data by a factor of 9 axially and 3 laterally. Average strains are calculated within a 9×3 window (approximately 0.127mm in depth and 0.308mm in lateral axis). A ramp-hold creep curve was measured from the time sequences of strain data frames at each pixel.

Two representative patients' data were studied. The creep curve data were fit to the plate compression model of Eq. (17). Eight randomly selected spatial points were analyzed, where points 1 – 4 are within the lesion and point 5 – 8 are outside the lesion. Figure 11 (a–b) gives representative creep curves for the benign and malignant lesions. The solid points are experimental creep data and the dotted lines are the fitting curves predicted by Eq (17). Models fit the data well, with $R^2 \sim 0.99$.

Statistical properties of viscoelastic parameters [E_0 , α , τ] were analyzed. Thirty small analysis regions 3×3 pixels were randomly selected by hand within the lesion and background regions of each patient image. In those regions, the average KVFD model parameters are estimated to give the mean values reported.

Tables 9 and 10 provide the best-fit KVFD model parameters from benign and malignant lesions, respectively. The average modulus of malignant lesions is an order of magnitude larger than that of benign lesions, which is consistent with the literature [16]. The fluidity parameter α in malignant lesions is higher than that of the benign lesions which also coincides with previous results [16].

5. Discussion and Conclusions

This paper developed and tested a theoretical framework for different ramp-hold indentation tests based on KVFD models. Combining basic indentation theory with fractional derivative analysis, the viscoelastic response of a variety of indentation tests (ramp-hold relaxation, ramp-loading creep and load-unload hysteresis) using different indenter shapes (spherical, conical-pyramidal and plate compression) were derived. The mathematical solutions were closed-form except for load-unload testing by a spherical indenter. Each was found to closely fit the appropriate measurement data recorded for a variety of soft viscoelastic media.

The main advantage of the KVFD model is its adaptability to broad applications. Including the entire data record length undoubtedly increases the fitting fidelity and reliability, as it seems to reduce the chances that the fitting algorithm will become trapped in local minima. Comparing the KVFD model results to those of the widely used Hertzian or Sneddon models, the KVFD model is able to use the ramp-on phase of the time series within the model fitting process to yield stable and consistent parametric estimates. These advantages of the KVFD model are found for macro-indentation and nano-indentation using AFM force mapping analysis.

KVFD model parameters form a concise viscoelastic space that provides consistent viscoelastic property measurements through accurate model fitting to experimental data. We examined indentation testing results under different indenter shapes (spherical versus plate), different loading scheme (ramp relaxation versus load-unload), and different scales (macro-versus nano-) for gel-cream samples. The results show that for different indenter shape and loading schemes, KVFD parameters agree within one standard deviation. KVFD parameters from different indentation scales are consistent with the same trend. These experiments yield consistent estimates of KVFD model parameters.

The KVFD model fit the AFM data on cells and tissue samples, suggesting these descriptions of viscoelastic responses could help us gain a clearer understanding of cell and tissue properties under a variety of microenvironments. It should be noted that some indentation responses from biologically active materials show a response to stimuli throughout the measurement, particularly at the initial force application. For those tissues, fitting mostly begins after transient response. KVFD models fit the response curves well after 0.1s. For AFM testing for gastric cells and breast ex vivo tissues, KVFD elasticity moduli show consistent results with that of the Sneddon model (see table 5 versus 6 and table 7 versus 8). KVFD modulus are $1/3 - 1/2$ of the elasticity modulus in Sneddon model for cells and $1/6$ for tissues. The clinical creep tests via ultrasonic imaging of breast tissues under plate compression show consistent results with results from the literature [16]. Fewer samples were studied in tissue and cell testing, which limits the statistical significance of these findings.

This paper summarizes results of applying the KVFD model to a broad range of experimental conditions relevant to the assessment of soft biological materials. Further work on cells and tissues will be explored to improve understanding of the role that the mechanical environment surrounding cells plays in promoting and inhibiting disease progression.

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Appendix I Solutions to ramp hold Spherical indentation tests

a) Ramp-hold relaxation

Combining (6-1) (7-1) and (8-1), substituting $n=3/2$, we obtain the ramp-hold relaxation response

$$P_r(t) = \frac{8\sqrt{R}}{3} \int_0^t \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \frac{d[h^{\frac{3}{2}}(\tau\tau)]}{d\tau\tau} d\tau\tau \quad (\text{I-1})$$

$$= \begin{cases} \frac{8\sqrt{R}}{3} \int_0^t \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot \frac{3}{2} v^{\frac{3}{2}} \tau\tau^{\frac{1}{2}} d\tau\tau & 0 \leq t \leq T_r \\ \frac{8\sqrt{R}}{3} \left(\int_0^{T_r} \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot \frac{3}{2} v^{\frac{3}{2}} \tau\tau^{\frac{1}{2}} d\tau\tau + \int_{T_r}^t 0 \cdot d\tau\tau \right) & t \geq T_r \end{cases}$$

1. For $0 \leq t \leq T_r$, let $\xi = \frac{\tau\tau}{t}$, and using $B(x, y) = \int_0^1 t^{x-1} (1-t)^{y-1} dt$, $\text{Re}(x) > 0$, $\text{Re}(y) > 0$, where $B(x, y)$ is a Beta function, we obtain

$$\begin{aligned} P_r(t) &= 4\sqrt{R}v^{\frac{3}{2}} \left(\frac{2}{3} E_0 t^{\frac{3}{2}} + \frac{\eta}{\Gamma(1-\alpha)} t^{\frac{3}{2}-\alpha} B\left(\frac{3}{2}, 1-\alpha\right) \right) \quad (\text{I-2}) \\ &= 4\sqrt{R}v^{\frac{3}{2}} E_0 t^{\frac{3}{2}} \left(\frac{2}{3} + \frac{1}{\Gamma(1-\alpha)} \left(\frac{t}{\tau}\right)^{-\alpha} B\left(\frac{3}{2}, 1-\alpha\right) \right) \end{aligned}$$

2. For $t \geq T_r$, let $\xi = \frac{\tau\tau}{T_r}$, and using $B(x; a, b) = \int_0^x t^{a-1} (1-t)^{b-1} dt$, where $B(x; a, b)$ is the incomplete Beta function, we obtain

$$\begin{aligned} P_r(t) &= 4\sqrt{R}v^{\frac{3}{2}} \left(\frac{2}{3} E_0 T_r^{\frac{3}{2}} + \frac{\eta}{\Gamma(1-\alpha)} T_r^{\frac{3}{2}-\alpha} B\left(\frac{T_r}{t}; \frac{3}{2}, 1-\alpha\right) \right) \quad (\text{I-3}) \\ &= 4\sqrt{R}v^{\frac{3}{2}} E_0 T_r^{\frac{3}{2}} \left(\frac{2}{3} + \frac{1}{\Gamma(1-\alpha)} \left(\frac{t}{T_r}\right)^{\frac{3}{2}} \left(\frac{T_r}{t}\right)^{-\alpha} B\left(\frac{T_r}{t}; \frac{3}{2}, 1-\alpha\right) \right). \end{aligned}$$

Combining (I-3) and (I-6), we have

$$P_{r,sphere}(t) = \begin{cases} 4\sqrt{R}v^{3/2}E_0t^{3/2}\left[\frac{2}{3} + \frac{(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{3}{2}, 1-\alpha\right)\right], & 0 < t \leq T_r \\ 4\sqrt{R}v^{3/2}E_0T_r^{3/2}\left[\frac{2}{3} + \frac{\left(\frac{t}{T_r}\right)^{3/2}\left(\frac{t}{\tau}\right)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{T_r}{t}, \frac{3}{2}, 1-\alpha\right)\right], & t \geq T_r \end{cases} \quad (I-4)$$

b) Ramp-load creeping

Combining (6-2)(7-2) and (8-2), we can derive the ramp-load creeping response

$$h_r^n(t) = \begin{cases} \frac{1}{K_G} \int_0^t \frac{v}{E_0} [1 - \mathbf{E}_\alpha(-(\frac{t-\xi}{\tau})^\alpha)] d\xi & 0 \leq t \leq T_r \\ \frac{1}{K_G} \int_0^{T_r} \frac{v}{E_0} [1 - \mathbf{E}_\alpha(-(\frac{t-\xi}{\tau})^\alpha)] d\xi + \int_{T_r}^t 0 \cdot J(t-\xi) d\xi & t > T_r \end{cases} \quad (I-5)$$

1. For $0 \leq t \leq T_r$, and using $\mathcal{L}\Gamma(z) = \Gamma(z+1)$, we can obtain

$$\int_0^t \mathbf{E}_\alpha\left(-\left(\frac{t-\xi}{\tau}\right)^\alpha\right) d\xi = t \mathbf{E}_{\alpha,2}\left(-\left(\frac{t}{\tau}\right)^\alpha\right) \quad (I-6)$$

Then we have

$$h_r^n(t) = \frac{vt}{K_GE_0} [1 - \mathbf{E}_{\alpha,2}\left(-\left(\frac{t}{\tau}\right)^\alpha\right)] \quad (I-7)$$

2. For $t > T_r$, we can obtain

$$\begin{aligned} \int_0^{T_r} \frac{v}{E_0} \left(1 - \mathbf{E}_\alpha\left(-\left(\frac{t-\xi}{\tau}\right)^\alpha\right)\right) d\xi &= \frac{v}{E_0} \left(T_r + (t - T_r) \mathbf{E}_{\alpha,2}\left(-\left(\frac{t-T_r}{\tau}\right)^\alpha\right) \right. \\ &\quad \left. - t \mathbf{E}_{\alpha,2}\left(-\left(\frac{t}{\tau}\right)^\alpha\right)\right) \end{aligned} \quad (I-8)$$

Then we have

$$h_r^n(t) = \frac{v}{K_G E_0} [T_r + (t - T_r) \mathbf{E}_{\alpha,2}(-(\frac{t-T_r}{\tau})^\alpha) - t \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)] \quad (\text{I-9})$$

Substituting $n=3/2$ and $K_G = \frac{8\sqrt{R}}{3}$ into (I-7) and (I-9), the spherical ramp-load creeping solution is

$$h_r^{\frac{3}{2}}(t) = \begin{cases} \frac{3}{8\sqrt{R}} \frac{kt}{E_0} (1 - \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)) & 0 \leq t \leq T_r \\ \frac{3}{8\sqrt{R}} \frac{k}{E_0} [T_r + (t - T_r) \mathbf{E}_{\alpha,2}(-(\frac{t-T_r}{\tau})^\alpha) - t \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)] & t \geq T_r \end{cases} \quad (\text{I-10})$$

It should be noted that the ramp-creeping solution (I-7) and (I-9) is applicable for other indenters when substitution of their corresponding K_G and n .

Appendix II Solutions to ramp hold conical-pyramidal indentation tests

a) Ramp-hold relaxation

Combining (6-1) (7-1) and (8-1), and substituting $n=2$ gives the ramp-hold relaxation response

$$P_r(t) = K_G \int_0^t \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \frac{d[h^2(\tau\tau)]}{d\tau\tau} d\tau\tau \quad (\text{II-1})$$

$$= \begin{cases} 2K_G \int_0^t \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v^2 \tau\tau d\tau\tau & 0 \leq t \leq T_r \\ 2K_G \left(\int_0^{T_r} \left(E_0 + \eta \frac{(t-\tau\tau)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v^2 \tau\tau d\tau\tau + \int_{T_r}^t 0 \cdot d\tau\tau \right) & t \geq T_r \end{cases}$$

As $P_1(t) = \int \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \cdot \xi d\xi = -(\frac{(t-\xi)^{1-\alpha}}{\Gamma(2-\alpha)} \xi + \frac{(t-\xi)^{2-\alpha}}{\Gamma(3-\alpha)})$, then we can easily obtain

$$P_{r, cone - pyramid}(t) =$$

$$\begin{cases} 2K_G E_0 v^2 t^2 \left(\frac{1}{2} + \frac{(t/\tau)^{-\alpha}}{\Gamma(3-\alpha)} \right) & 0 \leq t \leq T_r \\ 2K_G E_0 v^2 \left(\frac{T_r^2}{2} - \frac{(t-T_r)}{\Gamma(2-\alpha)} \left(\frac{t-T_r}{\tau} \right)^{-\alpha} - \frac{(t-T_r)^2}{\Gamma(3-\alpha)} \left(\frac{t-T_r}{\tau} \right)^{-\alpha} + \frac{t^2}{\Gamma(3-\alpha)} \left(\frac{t}{\tau} \right)^{-\alpha} \right) & t \geq T_r \end{cases}$$

(II-2)

b) Load-unload hysteresis

Combining (6-1)(7-1) and (8-3), we can obtain the load unload hysteresis response as

$$\begin{aligned} P_{hys, cone - pyramid}(t) &= K_G \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \frac{d[h^2(\xi)]}{d\xi} d\xi \\ &= \begin{cases} 2K_G \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v^2 \xi d\xi & 0 \leq t \leq T_r \\ 2K_G \left(\int_0^{T_r} \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v^2 \xi d\xi - \int_{T_r}^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) v^2 (2T_r - \xi) \cdot d\xi \right) & T_r \leq t \leq 2T_r \end{cases} \\ &= \\ &\begin{cases} 2K_G E_0 v^2 t^2 \left(\frac{1}{2} + \frac{(t/\tau)^{-\alpha}}{\Gamma(3-\alpha)} \right) & 0 \leq t \leq T_r \\ 2K_0 E_0 v^2 \left(\frac{t^2}{\Gamma(3-\alpha)} \left(\frac{t}{\tau} \right)^{-\alpha} - \frac{2T_r(t-T_r)}{\Gamma(2-\alpha)} \left(\frac{t-T_r}{\tau} \right)^{-\alpha} + \frac{(2T_r-t)^2}{2} \right) & T_r \leq t \leq 2T_r \end{cases} \end{aligned}$$

(II-3)

Appendix III Solutions to Ramp hold punch indentation tests

a) Ramp-hold relaxation

Combining (2-1) (6-1) and (8-1), we obtain the ramp-hold relaxation response

$$\sigma_r(t) = \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \frac{d[\varepsilon(\xi)]}{d\xi} d\xi \quad (\text{III-1})$$

$$= \begin{cases} \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v d\xi & 0 \leq t \leq T_r \\ \int_0^{T_r} \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v d\xi - \int_{T_r}^t 0 \cdot d\xi & t \geq T_r \end{cases}$$

As $P_2(t) = \int \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) d\xi = E_0 \xi - \eta \frac{(t-\xi)^{1-\alpha}}{\Gamma(2-\alpha)}$, we can easily derive that

$$\sigma_{r, punch}(t) = \begin{cases} \frac{\varepsilon_0 E_0 t}{T_r} \left[1 + \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)} \right] & 0 \leq t \leq T_r \\ \frac{\varepsilon_0 E_0}{T_r} \left[T_r - (t - T_r) \frac{((t - T_r)/\tau)^{-\alpha}}{\Gamma(2-\alpha)} + t \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)} \right] & t > T_r \end{cases} \quad (\text{III-2})$$

b) Load-unload hysteresis

Combining (2-2) (6-2) and (8-3), we obtain the load unload hysteresis response

$$\sigma_{hys, punch}(t) = \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \frac{d[\varepsilon(\xi)]}{d\xi} d\xi \quad (\text{III-3})$$

$$= \begin{cases} \int_0^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v d\xi & 0 \leq t \leq T_r \\ \int_0^{T_r} \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) \cdot v d\xi - \int_{T_r}^t \left(E_0 + \eta \frac{(t-\xi)^{-\alpha}}{\Gamma(1-\alpha)} \right) v d\xi & T_r \leq t \leq 2T_r \end{cases}$$

$$= \begin{cases} \frac{\varepsilon_0 E_0 t}{T_r} \left(1 + \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)} \right) & 0 \leq t \leq T_r \\ \frac{\varepsilon_0 E_0}{T_r} \left[(2T_r - t) - 2 \frac{(t - T_r)}{\Gamma(2-\alpha)} \left(\frac{t}{\tau} \right)^{-\alpha} + t \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)} \right] & T_r \leq t \leq 2T_r \end{cases}$$

Figure 1.
KVFD model

(a1)

(b1)

(a2)

(b2)

Figure 2.

Plots in (a1)–(a2) respectively give spherical ramp-hold relaxation response and load-unload response under different indentation depths. (a3) gives spherical ramp-hold creep response under different force loadings; (b1)–(b3) gives spherical ramp-hold relaxation, load-unload, and ramp-hold creep responses under different indenter diameters; (c1)–(c3) gives spherical

ramp-hold relaxation, load-unload and ramp-hold creep responses under different ramp times T_r ; (d1)–(d3) gives pyramidal-conical ramp-hold relaxation, load-unload and ramp-hold creep response under different indenter half angles.

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Figure 3.

Spherical indenter responses for media simulated over a range of model parameters. (a1)–(a3) are, respectively, ramp-hold relaxation responses, load-unload responses and ramp-hold creep responses under different E_0 values; (b1)–(b3) are the same responses for different α values; (c1)–(c3) are the responses for different τ values.

Figure 4.

Basic indentation testing protocols (a) ramp relaxation, (b) load-unload hysteresis, and (c) ramp-creep curve. The profile in the inset is applied and the larger plot is the response expected for viscoelastic media.

Figure 5.

KVFD fitting for ramp-relaxation and load-unload test data for Gel-Cream samples. (a) Eq. 9 (lines) are the best fit models to the ramp-relaxation data (different markers). (b) Eq. 10 (lines) are the best-fit models to the load-unload test data (different markers). In (a), experimental points are down sampled by a factor of 50 during the ramp phase and 100 during the holding phase for plotting clarity only. Similarly, in (b), experimental points are down sampled by a factor of 10.

Figure 6A

Figure 6B

Figure 6.

Figure 6A. (a) Spherical indentation and (b) plate compression test measurements (points) along with best-fit KVFD solutions (lines). The three curves correspond to the samples listed in the legend. The experimental curve is down sampled by a factor of 10 in the ramp phase and 100 in the holding phase to more clearly visualize the agreement between each model and the corresponding data [15].

Figure 6B. Comparison of estimated KVFD parameters from spherical indentation and plate compression tests. (a) Elastic modulus E_{0s} is measured by spherical indentation and E_{0p} is measured by plate compression; (b) Fractional derivative order α_s is found using spherical indentation and α_p by plate compression; (c) Time constant τ_s is found by spherical indentation and τ_p by plate compression [15].

Figure 7.

Ramp-hold nano-spherical indentation test measurement (points) along with best-fit KVFD solutions (lines). The four curves correspond to the different percentage gel-cream samples. The experimental curve is down sampled by a factor of 30 in the ramp phase and 100 in the holding phase.

Figure 8.

Viscoelastic parameters (a) modulus E_0 , (b) fractional order α and (c) time constant τ versus cream percentage are measured using nanoindentation methods. The error bars denote ± 1 sd. The trends in the cream-concentration data are similar to those found for macro-indentation methods (see Figs. 3 and 4 in [14]).

Figure 9.

Model fitting comparison of AFM load-unload data for gastric cells. AFM experimental data of gastric cancer cells without apigenin (a1) whole data (green circle marker) fit to KVFD model prediction (dash lines) and (b1) fit approach part (blue circle marker) of force-displacement curve to Sneddon model prediction (dash lines). AFM experimental data of gastric cancer cells with apigenin after 24 hours (a2) whole data (green circle marker) was fit to KVFD model prediction (dash lines) and (b2) fit approach part (blue circle marker) of force-displacement curve to Sneddon model prediction (dash lines).

Figure 10.

Model fitting comparison of AFM load-unload data for breast tissue. AFM experimental data of benign breast tissue (a1) whole data (green circle markers) fit to KVFD model prediction (line) and (b1) approaching part (blue circle markers) fit to Sneddon model prediction (line). AFM experimental data of malignant breast tissue (a2) whole data (green circle markers) fit to KVFD model prediction (line) and (b2) approaching part (blue circle markers) fit to Sneddon model prediction (line).

Figure 11.
Fitting of strain creep curve from (a) benign breast lesions and (b) malignant breast lesions

Table 1

Solutions to spherical indentation tests

Ramp-relax $P_{\text{sphere}}(t)$	$(9) \quad \begin{cases} 4\sqrt{R\nu}^{3/2}E_0t^{3/2}\left[\frac{2}{3} + \frac{(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{3}{2}, 1-\alpha\right)\right], & 0 < t \leq T_r \\ 4\sqrt{R\nu}^{3/2}E_0T_r^{3/2}\left[\frac{2}{3} + \frac{(t/T_r)^{3/2}(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{T_r}{t}, \frac{3}{2}, 1-\alpha\right)\right], & t \geq T_r \end{cases}$
Load-unload $P_{\text{phys,sphere}}(t)$	$(10) \quad \begin{cases} 4\sqrt{R\nu}^{3/2}E_0t^{3/2}\left[\frac{2}{3} + \frac{(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{3}{2}, 1-\alpha\right)\right], & 0 < t \leq T_r \\ 4\sqrt{R\nu}^{3/2}E_0T_r^{3/2}\left[\frac{2}{3} + \frac{(t/T_r)^{3/2}(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)}B\left(\frac{T_r}{t}, \frac{3}{2}, 1-\alpha\right)\right] - 4\sqrt{R\nu}^{3/2}E_0\int_{T_r}^t\left[1 + \frac{((t-\xi)/\tau)^{-\alpha}}{\Gamma(1-\alpha)}\right](2T_r-\xi)^{1/2}d\xi, & T_r < t \leq 2T_r \end{cases}$
Ramp creep $\frac{3}{2}h_r^2(t)$	$(11) \quad \begin{cases} \frac{3}{8\sqrt{R}}\frac{kt}{E_0}\left(1 - \mathbf{E}_{\alpha,2}\left(-\left(\frac{t}{\tau}\right)^\alpha\right)\right), & 0 \leq t \leq T_r \\ \frac{3}{8\sqrt{R}}\frac{k}{E_0}\left[T_r + (t - T_r)\mathbf{E}_{\alpha,2}\left(-\left(\frac{t-T_r}{\tau}\right)^\alpha\right) - t\mathbf{E}_{\alpha,2}\left(-\left(\frac{t}{\tau}\right)^\alpha\right)\right], & t \geq T_r \end{cases}$

where $B(x, y) = \int_0^1 t^{x-1}(1-t)^{y-1}dt$, $\text{Re}(x) > 0$, $\text{Re}(y) > 0$ is a complete beta function and $B(a; x, y) = \int_0^a t^{x-1}(1-t)^{y-1}dt$ for $x \in [0, 1]$ is an incomplete beta function.

Table 2

Solutions to conical-pyramidal indentation tests

Ramp Relax $P_{r,cone-pyram}(t)$	$\begin{cases} 2K_GE_0v^2t^2\left(\frac{1}{2} + \frac{(t/\tau)^{-\alpha}}{\Gamma(3-\alpha)}\right) & 0 \leq t \leq T_r \\ 2K_GE_0v^2\left(\frac{T_r^2}{2} - \frac{(t-T_r)}{\Gamma(2-\alpha)}\left(\frac{t-T_r}{\tau}\right)^{-\alpha} - \frac{(t-T_r)^2}{\Gamma(3-\alpha)}\left(\frac{t-T_r}{\tau}\right)^{-\alpha} + \frac{t^2}{\Gamma(3-\alpha)}\left(\frac{t}{\tau}\right)^{-\alpha}\right) & t \geq T_r \end{cases} \quad (12)$
Load-unload $P_{hys,cone-pyram}(t)$ $P_{r,cone-pyram}(t)$	$\begin{cases} 2K_GE_0v^2t^2\left(\frac{1}{2} + \frac{(t/\tau)^{-\alpha}}{\Gamma(3-\alpha)}\right) & 0 \leq t \leq T_r \\ 2K_GE_0v^2\left(\frac{t^2}{\Gamma(3-\alpha)}\left(\frac{t}{\tau}\right)^{-\alpha} - \frac{2T_r(t-T_r)}{\Gamma(2-\alpha)}\left(\frac{t-T_r}{\tau}\right)^{-\alpha} + \frac{(2T_r-t)^2}{2}\right) & T_r < t \leq 2T_r \end{cases} \quad (13)$
Ramp creep $h_r^2(t)$	$\begin{cases} \frac{1}{K_G} \frac{kt}{E_0} (1 - \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)) & 0 \leq t \leq T_r \\ \frac{1}{K_G} \frac{k}{E_0} [T_r + (t-T_r)\mathbf{E}_{\alpha,2}(-(\frac{t-T_r}{\tau})^\alpha) - t\mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)] & t \geq T_r \end{cases} \quad (14)$

Table 3

Solutions to plate indentation tests

Ramp-relax $\sigma_{\varepsilon,punch}(t)$	$\begin{cases} \frac{\varepsilon_0 E_0}{T_r} t (1 + \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)}), & 0 \leq t \leq T_r \\ \frac{\varepsilon_0 E_0}{T_r} (T_r - (t - T_r) \frac{((t - T_r)/\tau)^{-\alpha}}{\Gamma(2-\alpha)} + t \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)}), & t > T_r \end{cases} \quad (15)$
Load-unload $P_{hy,spyramp}(t)$	$\begin{cases} \frac{K_G E_0}{T_r} t (1 + \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)}) & 0 \leq t \leq T_r \\ \frac{K_G E_0}{T_r} [(2T_r - t) - 2 \frac{(t - T_r)}{\Gamma(2-\alpha)} (\frac{t}{\tau})^{-\alpha} + t \frac{(t/\tau)^{-\alpha}}{\Gamma(2-\alpha)}], & T_r < t \leq 2T_r \end{cases} \quad (16)$
Ramp creep $\varepsilon_f(t)$	$\begin{cases} \frac{\sigma_0}{E_0} \frac{t}{T_r} [1 - \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)], & 0 \leq t \leq T_r \\ \frac{\sigma_0}{E_0} \frac{1}{T_r} [T_r + (t - T_r) \mathbf{E}_{\alpha,2}(-(\frac{t - T_r}{\tau})^\alpha) - t \mathbf{E}_{\alpha,2}(-(\frac{t}{\tau})^\alpha)], & t > T_r \end{cases} \quad (17)$

KVFD model parameters estimated from different loading schemes. Moving towards the right, columns display data from samples with higher cream concentrations. The mean value for measurements over 3 days are shown and the error bars are ± 1 sd.

Table 4

Gel-Cream Samples		G5C5	G5C20	G5C30	G5C50
Ramp Relaxation	E_0 (Pa)	1654 $\pm$ 43	2364 $\pm$ 32	1855 $\pm$ 28	1789 $\pm$ 39
	α	0.084 $\pm$ 0.002	0.178 $\pm$ 0.001	0.236 $\pm$ 0.001	0.269 $\pm$ 0.005
	τ (s)	16.83 $\pm$ 7.21	21.86 $\pm$ 9.73	49.13 $\pm$ 15.78	70.20 $\pm$ 20.39
Load-unload	E_0 (Pa)	1641 $\pm$ 51	2351 $\pm$ 43	1832 $\pm$ 35	1778 $\pm$ 48
	α	0.082 $\pm$ 0.002	0.176 $\pm$ 0.003	0.231 $\pm$ 0.002	0.260 $\pm$ 0.006
	τ (s)	12.768 $\pm$ 5.93	19.382 $\pm$ 10.06	43.863 $\pm$ 20.37	63.731 $\pm$ 29.77

Table 5

KVFD parameters from gastric cancer cells. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

parameter	E_0 (Pa)	α	τ (s)
Control	15490.82 $\pm$ 108.62	0.71 $\pm$ 0.05	0.03 $\pm$ 0.005
APG	6498.92 $\pm$ 40.21	0.63 $\pm$ 0.04	0.01 $\pm$ 0.002

Table 6

Sneddon model parameters from gastric cancer cells. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

parameter	E (Pa)
Control	33020.61 $\pm$ 381.32
APG	16493.25 $\pm$ 216.15

Table 7

Best-fit KVFD model parameters from benign breast tissue. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

parameter	E_0 (Pa)	α	τ (s)
benign	3602.78 $\pm$ 330.13	0.010 $\pm$ 0.001	0.006 $\pm$ 0.0002
mal	552.04 $\pm$ 63.54	0.040 $\pm$ 0.01	0.012 $\pm$ 0.003

Table 8

best-fit Sneddon model parameters from malignant breast tissue. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

parameter	E (Pa)
benign	15235.14 $\pm$ 160.63
mal	3018.59 $\pm$ 292.11

Table 9

KVFD model parameters from a benign breast lesion measured in vivo using ultrasound. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

Benign lesion	within the lesion	outside the lesion
$E_0(Pa)$	6060.56 ± 59.08	5481.20 ± 14.86
α	0.16 ± 0.01	0.12 ± 0.01
$\tau(s)$	0.32 ± 0.10	0.24 ± 0.08

Table 10

KVFD model parameters from a malignant breast lesion measured in vivo using ultrasound. The mean value for measurements over 30 independent points are shown and the error bars are ± 1 sd.

malignant legion	within the lesion	outside the lesion
$E_0(Pa)$	58000.65 ± 3874.10	18338.74 ± 1136.40
α	0.35 ± 0.01	0.19 ± 0.01
$\tau(s)$	0.21 ± 0.16	4.27 ± 1.36