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# Strain rate viscoelastic analysis of soft and highly hydrated biomaterials

**A. Tirella,[1,2] G. Mattei,[1,3] A. Ahluwalia[1,2]**

[1]Research Centre "E. Piaggio", University of Pisa, Via Diotisalvi 2, 56124 Pisa, Italy
[2]National Research Council, IFC, Via Moruzzi 1, 56122 Pisa, Italy
[3]Department of Civil and Industrial Engineering, University of Pisa, Largo Lucio Lazzarino 1, 56126 Pisa, Italy

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**Abstract:** Measuring the viscoelastic behavior of highly hydrated biological materials is challenging because of their intrinsic softness and labile nature. In these materials, it is difficult to avoid prestress and therefore to establish precise initial stress and strain conditions for lumped parameter estimation using creep or stress-relaxation (SR) tests. We describe a method (ἐM or epsilon dot method) for deriving the viscoelastic parameters of soft hydrated biomaterials which avoids prestress and can be used to rapidly test degradable samples. Standard mechanical tests are first performed compressing samples using different strain rates. The dataset obtained is then analyzed to mathematically derive the material's viscoelastic parameters. In this work a stable elastomer, polydimethylsiloxane, and a labile hydrogel, gelatin, were first tested using the ἐM, in parallel SR was used to compare lumped parameter estimation. After demonstrating that the elastic parameters are equivalent and that the estimation of short-time constants is more precise using the proposed method, the viscoelastic behavior of porcine liver was investigated using this approach. The results show that the constitutive parameters of hepatic tissue can be quickly quantified without the application of any prestress and before the onset of time-dependent degradation phenomena. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 3352–3360, 2014.

**Key Words:** soft tissues, viscoelasticity, mechanical properties, strain rate, liver

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## INTRODUCTION

Measuring and characterizing the mechanical properties of soft materials are important for understanding, monitoring, and predicting their performance and responses in specific conditions. Highly hydrated and degradable materials (e.g., hydrogels and biological soft tissues) are particularly challenging due to their shape, softness, and labile nature.

Soft tissues and hydrogels are typical biphasic materials in which a solid network is swollen and surrounded by water: the solid part is generally responsible for their elasticity, whereas viscosity arises both from network mobility and the contribution of water and other molecules in the aqueous solution.[1] It is known that these materials, and their mechanical properties, are highly sensitive to environmental conditions and prestress (e.g., they may degrade, change water content over time, or deform irreversibly under small preloads). Indeed, the identification of a reliable method of testing which does not alter the native material properties before or during testing, and can thus be used to derive a unique and meaningful set of constitutive parameters, is still the subject of debate.

Often the mechanical characterization of hydrated materials using compressive tests is limited to quasistatic loading conditions.[2] However, since the time-dependent response is crucial in understanding the behavior of viscoelastic materials, time variant or dynamic analysis enable a more complete description of material properties. In this direction, Trexler et al.[3] describe the effects of high strain rates on soft biomaterials, whereas Pervin et al.[4] report on the time response of liver, highlighting the effect of different strain rates on the resultant measured stiffness.

Probably the most well-known methods for characterization of viscoelastic materials are creep and stress relaxation (SR). After the application of a step force or deformation, strain–time or stress–time data are used to derive viscoelastic parameters from constitutive models. The simplest parameter estimation models are based on the assumption that the applied stimulation is a pure Heaviside step. Creep and SR tests, such as those proposed by Stammen et al.,[5] provide precise and complete information on the time-dependent response of stable viscoelastic specimens. A number of alternative mechanical testing methods based on indentation have been proposed in the last decade, many of them focusing on microindentation systems using atomic force microscopy.[6,7] Here, step and ramp loads or deformations have been used to identify the constitutive properties

**Correspondence to:** A. Tirella; e-mail: a.tirella@ifc.cnr.it

of soft and wet materials through the use of theoretical relationships between applied load, penetration depth and the geometry of the indenter. Mattice et al.[8] and Cheng et al.[9] demonstrate the use of microindentation for creep and SR, showing that viscoelastic constants can be derived using lumped parameter models for both polymeric hydrogels and soft tissues. However, indentation methods are confined to probing a highly localized viscoelastic half space, thus derived parameters typically characterize small surface regions, rather than the bulk.

More complex methods such as dynamic mechanical analysis (DMA) also enable parameter estimation of hydrated biomaterials. A frequency sweep of stress or strain is applied to the material under investigation, which must remain in contact with the oscillating platen to ensure repeatable data collection. For instance Marchesseau et al.[10] performed shear dynamic frequency sweep experiments on hepatic tissue in the range 0.1/4 Hz. Liver constitutive parameters were estimated fitting the measured frequency-dependent shear dynamic modulus to a generalized Maxwell (GM) model. Alternatively, the time dependency of stress–strain behavior of these materials can be investigated through cyclic loading. Bergstrom and Boyce[11] have proposed a constitutive model which takes into account different hypothesized phenomena in the macromolecular network of viscoelastic polymers whereby estimated parameters are used to fit cyclic loading curves.

The constitutive models used for parameter extraction necessitate the establishment of a well-defined and mathematically tractable stimulus (e.g., a step or sinusoidal load or deformation, defined indenter geometry) and controlled initial configuration (no prestress or strain applied on tested samples). All of the above testing methods are based on a priori assumptions about the nature of the applied mechanical stimulus or initial conditions. Consequently, in the context of soft, hydrated, and labile biomaterials, they are often unsatisfactory, particularly as regards the initial conditions and the experimental set-up.

To address these issues, we have developed a new method (the $\dot{\varepsilon}$M or epsilon dot method), based on the application of different compressive strain rates for the derivation of material viscoelastic parameters from constitutive equations. The $\dot{\varepsilon}$M represents an alternative to classic SR and creep for the derivation of lumped parameters of soft and labile materials and is characterized by (i) the absence of prestress, (ii) a well-defined compressive stimulus, and (iii) the short duration of the testing phase. These three features enable the unique and accurate identification of model inputs and the preservation of material properties during the tests. Here, we describe the method and its application to three different materials. First, polydimethylsiloxane (PDMS), a well-characterized viscoelastic polymer, was used to validate the approach. Gelatin was then used to demonstrate its advantages over classical SR tests for the evaluation of viscoelastic parameters of soft, hydrated, and labile biopolymers.

Gelatin, a derivative of collagen commonly used in many biomedical applications, was chosen because it is highly hydrated but stiff enough to withstand small prestresses without undergoing plastic deformation, and also possesses a biphasic viscoelastic network similar to that of soft biological tissue. Moreover it has been widely used in viscoelastic and strain rate dependence reports, and its properties are well documented.[1,12–15]

To assess the performance of the $\dot{\varepsilon}$M in characterizing biological samples, we also tested hepatic tissue from porcine liver. There is great interest in the correlation between the mechanical properties of hepatic tissue and liver fibrosis, although, as shown by Marchesseau et al.,[10] elastic moduli reported for healthy liver range over two orders of magnitude, even in quasistatic conditions. Ocal et al.[16] correctly state that this is due to the labile nature of the tissue as well as to the measurement conditions, in which hydration and stress may vary. In fact, given the extremely soft and viscoelastic nature of this tissue, the measurement itself can alter the measured mechanical properties of the tissue, particularly in the case of high strains[17] and long testing times.[16]

In summary, in this work the main objective is to demonstrate that the $\dot{\varepsilon}$M can be used to derive the properties of viscoelastic materials and biological tissues by global parameter estimation and fitting of data collected at different strain rates using the same equipment employed for SR and creep tests. Moreover we show that the method is particularly suited to testing soft materials, which may degrade or alter over time.

## MATERIALS AND METHODS

### Sample preparation

PDMS Sylgard 184 was prepared in accordance with the manufacturer's specifications. To obtain samples with flat faces, the prepolymer solution was cast into cubic molds (10 mm sides) and cured at 60°C; after the curing process, samples were removed from the molds and ready for testing.

Gelatin samples were prepared using a 5% (w/v) gelatin solution. The solution was obtained dissolving gelatin type-A (300 bloom strength) in deionized water, and stirred at 50°C for 2 h. Gelatin solution was gently poured into cylindrical (60 mm diameter and 10 mm height) and cubic (10 mm sides) molds, respectively, for SR and $\dot{\varepsilon}$M tests. Samples were gelled at room temperature and stored at 4°C until use. The large difference in cross-section between the two samples was necessary to ensure the detection of a measurable force by the load cell throughout the duration of SR tests.

Fresh porcine livers from 1 year old healthy swine were collected as a slaughter by-product and frozen at −20°C until use. Cubic samples (about 10 mm sides) were excised from frozen liver to have regular shapes with parallel loading surfaces. Attention was dedicated to avoid the capsular connective tissue (i.e., Glisson's capsule) and macroscopic vasculature while cutting the sample. To ensure a repeatable sample testing state, liver samples were equilibrium swollen in phosphate-buffered saline 1× at 4°C and then brought to

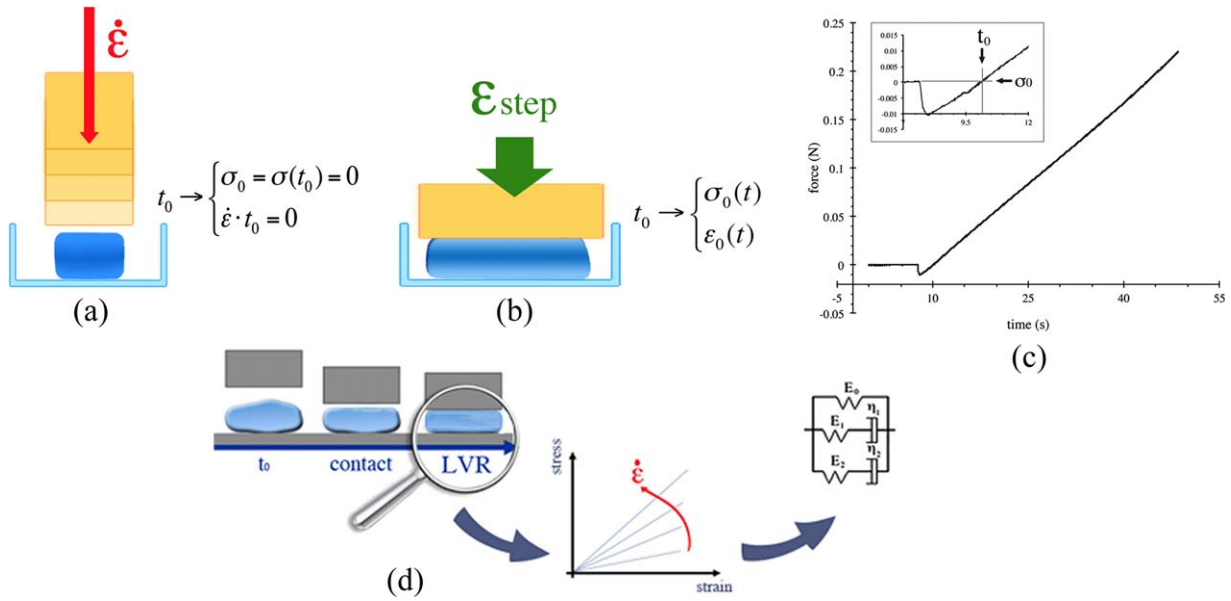


**FIGURE 1**. Experimental set-up and initial conditions for: (a) $\dot{\varepsilon}$M test, showing zero-stress (no contact between sample and plate) and (b) SR test, showing prestress (plate in contact with the sample). c, Details of a typical force–time recording during a $\dot{\varepsilon}$M test. d, The $\dot{\varepsilon}$M workflow: from the testing phase to the derivation of viscoelastic parameters. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

room temperature before testing.[18] Liver samples were considered to be mechanically isotropic.[4]

Before mechanical testing, sample dimensions were carefully measured using a caliper (with a resolution of 0.050 mm) averaging measurements made at three different points.

### Mechanical tests

Compressive mechanical tests were performed imposing different strain rates using the twin column ProLine Z005 testing machine (Zwick Roell) equipped with a 10- and 100-N load cell (the former used for testing gelatin and liver, the latter for PDMS samples) at room temperature. Gelatin and liver samples were tested partially immersed in water to preserve their hydration. For the $\dot{\varepsilon}$M, each test was performed using a starting configuration with the upper plate placed near the sample but not in contact [as shown in Fig. 1(a)] necessary to guarantee known initial conditions. In the case of PDMS and gelatin, stress–strain time series were collected compressing samples ($n = 3$) using at least five different strain rates ($\dot{\varepsilon}$, ranging from 0.01 to 5% $s^{-1}$ and with $\varepsilon$ up to 1%). Using the lowest strain rate value the test lasts about 2 min.

Comparative SR tests ($n = 5$) were performed using the same instrument and load cell. The upper plate was placed just in contact with the sample [Fig. 1(b)], then a 1% strain (comprised within the material's linear range, see Evaluation of initial stress and strain conditions section) was applied on tested samples and the force was monitored for up to 20 min.

Three different compressive strain rates (1, 2, and 3% $s^{-1}$) were used for hepatic tissue. Note that SR could not be performed on liver samples because their heterogeneous nature did not allow the isolation of uniform samples large enough to withstand significant prestress on tested specimens when in contact with the upper plate.

### Evaluation of initial stress and strain conditions

Using the initial configuration shown in Figure 1(a), no prestresses acts on the tested sample, guaranteeing a zero stress initial condition. Once the plate starts to move, force and displacement data are recorded over time. As the plate approaches the sample, a negative force is recorded, due to water/plate surface tension. The instant in which the plate contacts and starts to stress the material (i.e., $t_0$) is easily recognized in Figure 1(c) as the point at which the force–time curve crosses the abscissa. This initial time was defined as $t_0 = 0$. Datasets with unique determination of the initial strain and stress, respectively, $\dot{\varepsilon} \cdot t_0$ and $\sigma_0 = \sigma(t_0) = 0$, were obtained and analyzed first as stress–strain curves, allowing establishment of the linear viscoelastic region (LVR, the region of small deformations in which the viscoelastic parameters remain constant). Then stress–time data series (corresponding to the identified LVR) were used to estimate material viscoelastic parameters as described in the following section and depicted in Figure 1(d).

### Lumped parameter estimation

To determine the viscoelastic parameters of materials, constitutive equations for different lumped parameter models [i.e., Maxwell-type standard linear solid (SLS) and GM model] were derived using Laplace transforms. In the case of the $\dot{\varepsilon}$M, equations were expressed considering a constant

compressive strain rate ($\dot{\varepsilon}$) acting on tested samples, whereas SR equations were derived as step responses to compressive strain ($\varepsilon_{step}$). Both $\dot{\varepsilon}$ and $\varepsilon_{step}$ represent experimental values imposed during mechanical tests.

Inverse Laplace transformations were used to derive viscoelastic parameters (i.e., $E_i$ and $\eta_j$ depending on the viscoelastic model used) for both testing methods. The general form of resultant stress–time relations were expressed as shown in the following equations, respectively, for $\dot{\varepsilon}$M and SR. The equations obtained were used to fit the experimental stress–time datasets. A more complete mathematical derivation is given in Appendix A.

$$\sigma(t)=\dot{\varepsilon}\cdot f(E_i,\eta_j,t) \tag{1}$$

$$\sigma(t)=\varepsilon_{step}\cdot f(E_i,\eta_j,t) \tag{2}$$

***Three-parameter models: Maxwell-type SLS.*** Three-element models are often used to describe viscoelastic materials combining springs and dashpots in different configurations. They can be distinguished in two classes: SLS and standard linear fluid models.[19–21] Since both materials used in this work have well-defined shapes and a LVR, the Maxwell-type SLS model was chosen as the simplest mechanical equivalent of tested materials. The differential constitutive equation describing a Maxwell-type SLS is expressed in Eq. (3), whereas $\dot{\varepsilon}$M and SR stress–time relationships are, respectively, Eqs. (4) and (5).

$$\dot{\sigma}+\frac{E_1}{\eta_1}\sigma=(E_0+E_1)\dot{\varepsilon}+\frac{E_0E_1}{\eta_1}\varepsilon \tag{3}$$

$$\sigma(t)=\dot{\varepsilon}\cdot\left(\eta_1-\eta_1 e^{-\frac{E_1t}{\eta_1}}+E_0t\right) \tag{4}$$

$$\sigma(t)=\varepsilon_{step}\cdot\left(E_0+E_1e^{-\frac{E_1t}{\eta_1}}\right) \tag{5}$$

***Five-parameter model: GM.*** Most viscoelastic materials possess more than one relaxation time, and it is generally accepted that models having more than three elements better describe the response of viscoelastic materials in time. Therefore to have a more complete analysis of material mechanical behavior, increasingly complex lumped parameter models are generally used. These models combine more elements disposed in series or in parallel, and are known as GM or Kelvin models. In general, each unit is composed of a spring and a dashpot, defining a relaxation time typical of material bulk properties. A five-parameter GM model [Eq. (6)] was used to evaluate whether gelatin possess more than one relaxation time. Stress–time relationships for $\dot{\varepsilon}$M and SR are expressed in Eqs. (7) and (8), respectively.

$$\begin{aligned}\ddot{\sigma}+\frac{E_1\eta_2+E_2\eta_1}{\eta_1\eta_2}\dot{\sigma}+\frac{E_1E_2}{\eta_1\eta_2}\sigma=E_0E_1E_2\ddot{\varepsilon}\\ +E_0\left(\frac{E_1\eta_2+E_2\eta_1}{\eta_1\eta_2}\right)\dot{\varepsilon}+\frac{E_0E_1E_2}{\eta_1\eta_2}\varepsilon\end{aligned} \tag{6}$$

$$\sigma(t)=\dot{\varepsilon}\cdot\left(E_0t+\eta_1\left(1-e^{-\frac{E_1}{\eta_1}t}\right)+\eta_2\left(1-e^{-\frac{E_2}{\eta_2}t}\right)\right) \tag{7}$$

$$\sigma(t)=\varepsilon_{step}\cdot\left(E_0+E_1e^{-\frac{E_1}{\eta_1}t}+E_2e^{-\frac{E_2}{\eta_2}t}\right) \tag{8}$$

Although different and more complex viscoelastic models can be used to determine lumped parameters, it has to be underlined that the higher the number of elements to estimate, the more inputs are required by the model. Since our objective was the validation of this new method, more complex models with more than five elements were not considered.

### Data analysis and fitting

Once the LVR of each material was identified, a dataset with all stress–time series as function of applied strain rate was globally fitted performing chi-square minimization in a combined parameter space. Viscoelastic parameters were shared during the fitting process using a $\dot{\varepsilon}$M fitting toolbox: a collection of viscoelastic models was implemented in OriginLab (Northampton) for this analysis. SR experimental data were fitted using the same models used for $\dot{\varepsilon}$M (Matlab) to derive the viscoelastic parameters. Comparisons between parameter values were made using the Student's $t$ test. Significance was set at $p < 0.05$.

## RESULTS

To validate the proposed method, PDMS, an elastomer with well-defined and documented viscoelastic parameters,[22,23] was first tested. Experimental datasets derived from $\dot{\varepsilon}$M and SR [plotted, respectively, in Fig. 2(a,b)] were analyzed and the resulting lumped parameters were compared. As expected, stress–strain plots of PDMS samples derived using different compressive strain rates do not differ significantly [Fig. 2(c)] and minimal hysteresis was observed during recovery [Fig. 2(d)], indicating that PDMS exhibits essentially elastic behavior, with a low viscous component.

The apparent elastic moduli ($E_{app}$) measured from the slopes of the stress–strain curves (Table I) have slight variations with compressive strain rate. For this reason, stress–time PDMS datasets were analyzed using a Maxwell-type SLS model (using a LVR of 1% strain). The instantaneous and equilibrium elastic moduli ($E_{inst}$ and $E_{eq}$, respectively) obtained using the two methods do not differ significantly ($p = 0.55$ for $E_{inst}$ and $p = 0.90$ for $E_{eq}$) as reported in Table II. However, the relaxation time (and its corresponding standard error) estimated using SR is much higher than that obtained using $\dot{\varepsilon}$M. This discrepancy is likely due to the fact that the rise time of the step force in SR is of the order of 1 s, as consequence short relaxation times are not detected during SR tests. This is also confirmed by evaluation of residuals between experimental and fitted data during the first few seconds of the tests, which were higher for all SR tests.

In view of the fact that the compressive strain rate tests all last less than 2 min and no prestress is applied to the material, we also tested a hydrogel and labile material: gelatin. SR was used again as control to better assess the

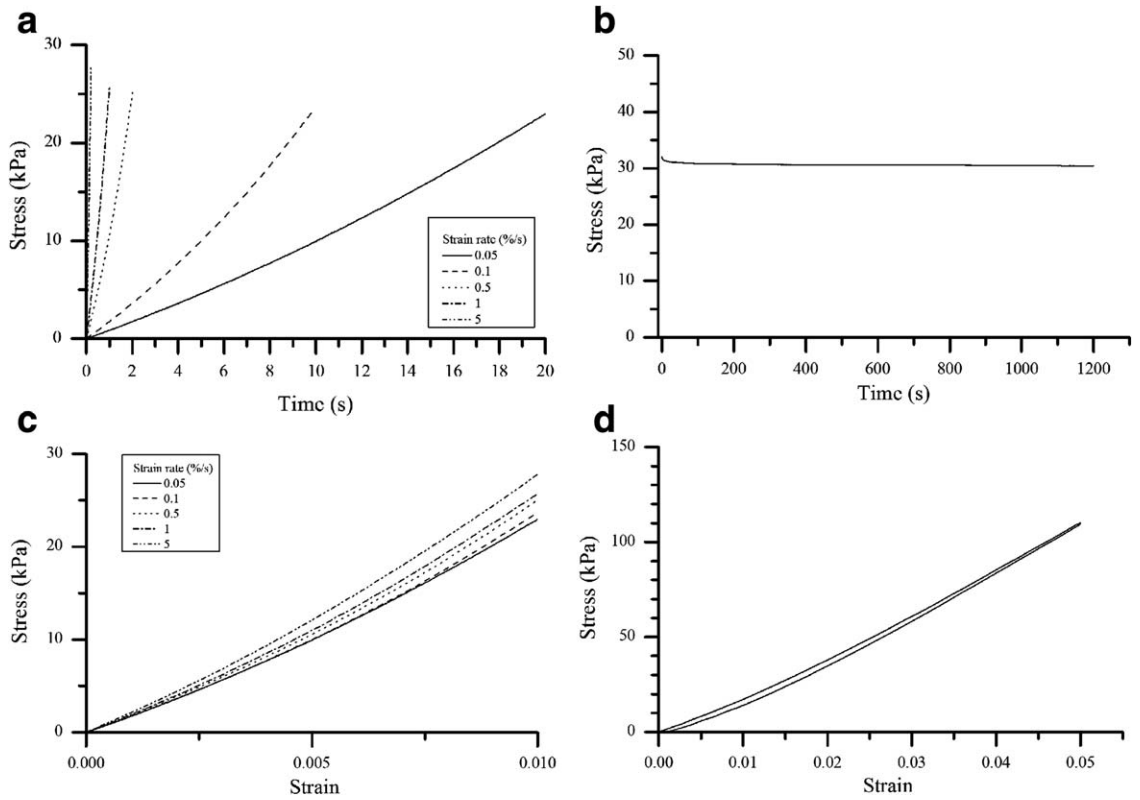


**FIGURE 2**. Experimental data of PDMS samples used for: (a) $\dot{\varepsilon}$M, (b) SR analysis, (c) material behavior as function of strain rate, and (d) material recovery at a compression rate of 0.5% $s^{-1}$.

potential of this method. At the 5% (w/v) concentration used, gelatin forms thermally reversible gels undergoing a sol–gel transition at around 28°C. When the gelatin samples are tested at room temperature, they tend to a partial gel–sol transition, particularly in case of long experiments. Consequently, we hypothesize that testing gelatin with fast protocols will result in more accurate measurements of material bulk properties; on the contrary, longer experimental procedures at room temperature could introduce errors in the measurement of viscoelastic parameters owing to a state transition.

In Figure 3(a) gelatin stress–time data series for compressive tests varying the strain rate are shown, whereas Figure 3(b) reports data obtained from the SR experiment. As shown, the SR tests were terminated after about 10 min since zero force was recorded (due to the gel–sol transition of tested samples).

Gelatin's viscoelastic behavior is clearly depicted in Figure 3(c) and confirmed by values reported in Table I, with a strong dependence between apparent elastic modulus and strain rate. Moreover, the hysteresis plot of tested samples [shown in Fig. 3(d)] highlights the labile nature of the material. During the 15-min-long hysteresis test, gelatin visibly undergoes a gel–sol transition resulting in plastic deformation. On the basis of these results, two viscoelastic models were chosen to fit the gelatin datasets: the Maxwell-type SLS model and the GM model. For both datasets the $R^2$ value for the Maxwell SLS was lower than that of the GM model, indicating that the latter model better represents gelatin. Both $E_{inst}$ and $E_{eq}$ for the two methods are similar ($p > 0.6$), but the errors are again consistently higher in SR estimated parameters. However SR fitting gives much longer relaxation

**TABLE I. Apparent Compressive Elastic Modulus of (a) PDMS, (b) Gelatine, and (c) Liver Samples**

| $\dot{\varepsilon}$ (% $s^{-1}$) | PDMS (MPa) | Gelatin (kPa) | Liver (kPa) |
|---|---|---|---|
| 0.01 | – | 3.19 ± 0.14 | – |
| 0.05 | 2.09 ± 0.08 | 4.54 ± 0.09 | – |
| 0.1 | 2.18 ± 0.01 | 5.70 ± 0.20 | – |
| 0.5 | 2.27 ± 0.05 | 8.98 ± 0.15 | – |
| 1 | 2.40 ± 0.02 | 10.86 ± 0.13 | 0.92 ± 0.02 |
| 2 | – | – | 1.15 ± 0.04 |
| 3 | – | – | 1.33 ± 0.05 |
| 5 | 2.50 ± 0.10 | – | – |

Data are derived from the LVR region of stress–strain curves varying the strain rate in the range of 0.01 and 5% $s^{-1}$.

**TABLE II. Viscoelastic Parameters Derived From Fitting of Experimental Curves of PDMS: Comparison Between $\dot{\varepsilon}$M and SR**

| | $\dot{\varepsilon}$M | SR |
|---|---|---|
| $E_{inst}$ (MPa) | 2.55 ± 0.04 | 2.14 ± 0.88 |
| $E_{eq}$ (MPa) | 2.14 ± 0.01 | 2.09 ± 0.58 |
| $\tau_1$ (s) | 0.66 ± 0.25 | 19.98 ± 12.78 |
| $R^2$ | 0.98 | 0.89 |

**TABLE III. Viscoelastic Parameters Derived From Fitting of Experimental Curves of Gelatin Hydrogels Using Maxwell SLS and GM Lumped Parameter Models: Comparison Between ε̇M and SR**

| | ε̇M | | SR | |
|---|---|---|---|---|
| | Maxwell SLS | Generalized Maxwell | Maxwell SLS | Generalized Maxwell |
| $E_{inst}$ (kPa) | 11.23 ± 0.45 | 13.24 ± 0.53 | 14.32 ± 1.46 | 14.14 ± 1.72 |
| $E_{eq}$ (kPa) | 2.43 ± 0.10 | 1.64 ± 0.07 | (71.72 ± 6.57) × $10^{-3}$ | 1.29 ± 0.71 |
| $\tau_1$ (s) | 4.85 ± 0.19 | 0.82 ± 0.03 | 158.59 ± 126.34 | 45.10 ± 29.43 |
| $\tau_2$ (s) | – | 15.07 ± 0.60 | – | 152.01 ± 19.77 |
| $R^2$ | 0.92 | 0.95 | 0.89 | 0.91 |

times compared with the ε̇M (Table III), and again residuals of the first few seconds of the SR tests were higher.

Having established that the ε̇M can be used to quickly test hydrated and labile materials, so without risk of material degradation, it was used to evaluate the viscoelastic parameters of liver. Hepatic tissue is very soft and quickly degrades at room temperature; the measurement and characterization of its mechanical properties is known to present several challenges. To use ε̇M, samples were compressed with different strain rates [each test ends in <3 s, Fig. 4(a)] within material LVR (3%). The viscoelastic behavior of liver is reflected in the increase in apparent elastic modulus with applied strain rate [Fig. 4(b) and Table I].

Note that $\tau_1$ and $\tau_2$ of the GM model are equivalent and correspond to the relaxation time ($\tau_1$) obtained using the Maxwell model (Table IV), suggesting that one Maxwell arm is enough to represent the viscoelastic behavior of the tested samples. As explained in Evaluation of initial stress and strain conditions section, due to the nature of hepatic tissue, SR tests could not be performed with our most sensitive load cell. However the fact that the estimated equilibrium modulus is null suggests that liver samples deprived of their connective capsule have a fluid-like behavior in accordance with the results obtained by Liu and Bilston.[24]

## DISCUSSION

Here, we propose an alternative method to SR and creep for the derivation of viscoelastic parameters of hydrated and degradable soft materials. Tests can be performed rapidly in hydrated conditions, and with defined initial and boundary conditions, simply applying a series of constant compressive

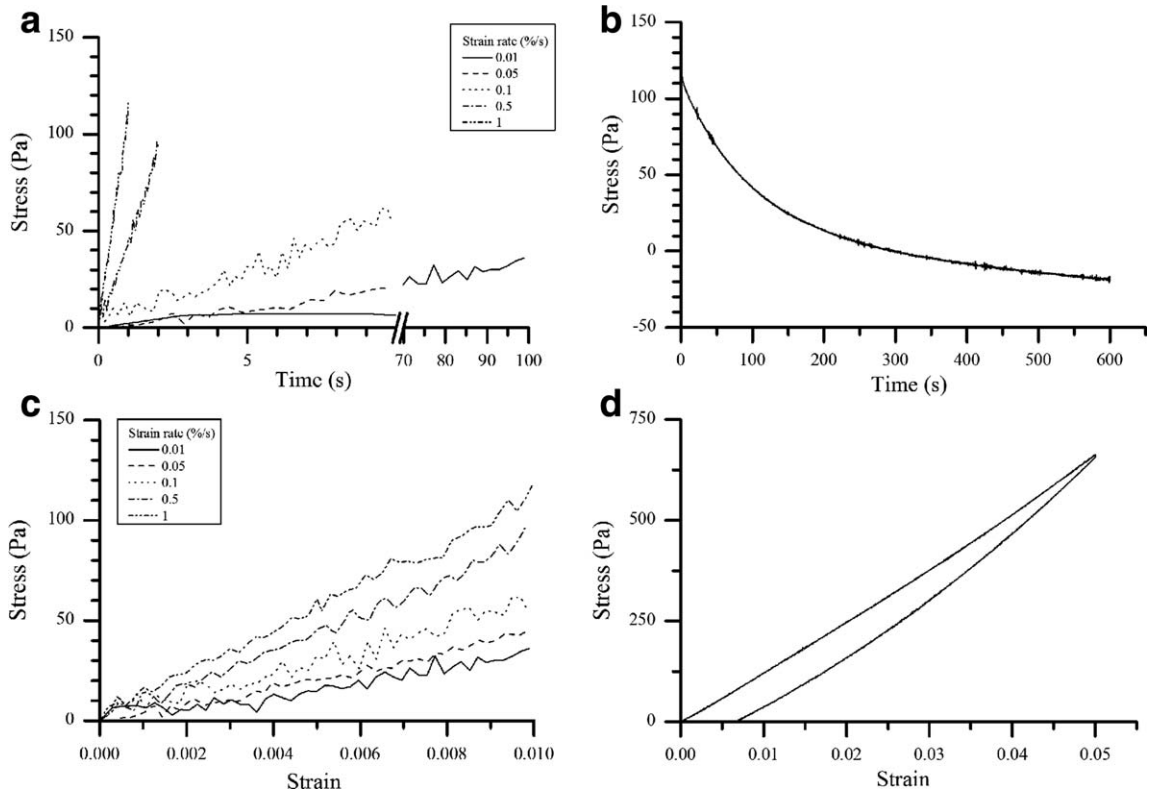


**FIGURE 3.** Experimental data of gelatin samples used for: (a) ε̇M, (b) SR analysis, (c) material behavior as function of strain rate, and (d) material recovery at a compression rate of 0.5% $s^{-1}$.

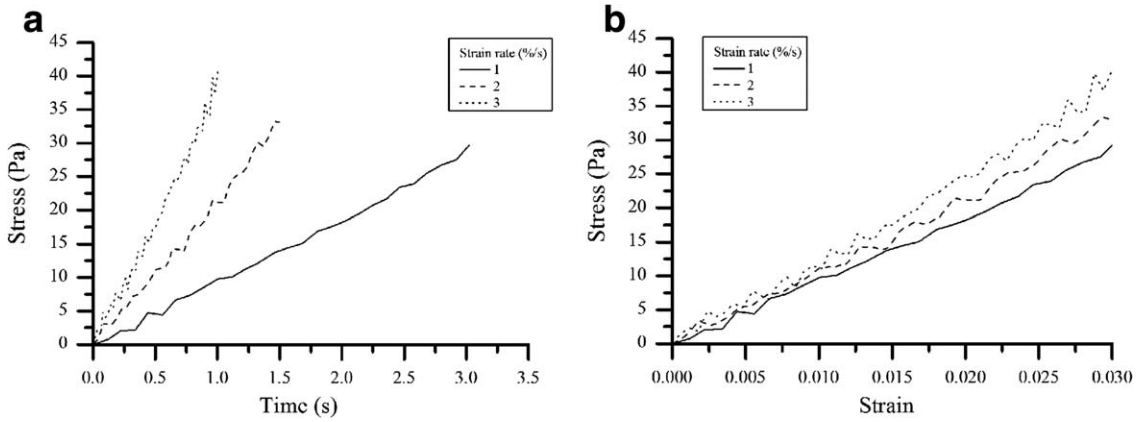


**FIGURE 4.** Stress–time (a) and stress–strain (b) plots of liver samples obtained from compressive tests on liver samples at different strain rates (i.e., 1, 2, and 3% $s^{-1}$).

strain rates. Experimental datasets are then used to derive viscoelastic parameters from constitutive equations.

Although strain rate tests have been used to characterize materials such as gelatin[25] and liver,[4] as far as we know this is the first application of such tests to derive material properties in the form of constitutive parameters. In fact the ε̇M method comprises both the testing phase, in which a range of compressive strain rates are applied to samples, as well as the successive mathematical derivation of viscoelastic models (i.e., expressed as a function of elastic and viscous constants, stress, strain, and their time derivatives) estimating their lumped parameters from analyses of LVR stress–time data sets [Fig. 1(d)].

First, we tested and compared the obtained parameters from both ε̇M and SR tests of a well-known, fairly rigid, and stable elastomer (PDMS). The same elastic modulus was estimated using a simple SLS model, but SR overestimates the viscous component of this essentially elastic material. The short characteristic relaxation time obtained using ε̇M is within the range of values suggested by Lin et al.[23] using DMA (i.e., 0.165 and 5 s compared with our 0.66 s). Since the same apparatus was used for both tests, this result underlines the higher accuracy of the ε̇M with respect to SR in determining short-time constants.

Hydrated gelatin samples were then tested to compare the performance of ε̇M and SR in parameter estimation of a soft and hydrated material. Using a five parameter GM model, the instantaneous and equilibrium elastic moduli obtained are similar, whereas the estimated relaxation times are considerably longer in SR analysis. Since the duration of strain rate compressive tests is of the order of a few tens of seconds at the most, we can assume that gelatin's state does not change during these tests. On the other hand, SR tests are longer: as shown by the derived SR time constants and measured force, it is reasonable to assume that a partial gel–sol transition occurs within the samples during the 10 min of applied stress. For this reason derived time constants from SR analysis likely represent the state transition rather than the intrinsic viscoelastic properties of the material. Moreover, the overestimation of gelatin stiffness derived from SR analysis can be explained considering the initial configuration of experiments: the starting contact probe/material can cause a small, but significant, prestress on soft samples, causing unequivocal errors in viscoelastic parameter derivation.

Finally, we show that the viscoelastic properties of hepatic tissue can be quickly and reproducibly characterized using the proposed method, minimizing any risk of degradation which may lead to erroneous parameter estimation. Fitting the dataset to Eq. (7) gives an $R^2$ value of 0.96, and the instantaneous elastic modulus of 1300 Pa is in good agreement with that obtained by Raghunathan et al.[26]

We underline that ε̇M cannot be considered as a replacement for SR, which has clear advantages for stable and stiffer materials: experimentally it requires a larger number of tests with respect to SR, and therefore may not be suitable for expensive or rare samples which cannot be used more than once. Furthermore, the number of lumped parameters that can be precisely identified will depend on the number of strain rates used, and the range of strain rates employed need to be established on the basis of predicted relaxation times. A single, long SR test on the other hand will enable derivation of multiple parameters.

In conclusion, the ε̇M method can be considered as a suitable alternative to creep and SR for the derivation of a unique set of viscoelastic parameters for soft, hydrated, and degradable materials which cannot be subject to long testing times and which will deform or stiffen significantly under low prestress.

**TABLE IV. Viscoelastic Parameters Derived From Fitting of Experimental Curves of Liver Using Maxwell SLS and GM Lumped Parameter Models**

| | Maxwell SLS | Generalized Maxwell |
|---|---|---|
| $E_{inst}$ (kPa) | 1.30 ± 0.07 | 1.30 ± 0.07 |
| $E_{eq}$ (kPa) | 0 | 0 |
| $\tau_1$ (s) | 4.22 ± 0.32 | 4.26 ± 0.35 |
| $\tau_2$ (s) | – | 4.28 ± 0.43 |
| $R^2$ | 0.96 | 0.96 |

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## APPENDIX A

In general, lumped parameter models combine pure springs and dashpots in different configurations to describe material viscoelastic behavior. The Maxwell-type SLS is one of the commonest viscoelastic models: it is composed of a pure spring ($E_0$) in parallel with a Maxwell arm, which consists of a spring ($E_1$) in series with a dashpot ($\eta_1$). However, the most general form of linear viscoelastic model is an extension of the simple Maxwell SLS model called GM model (Fig. A1). The general model possesses a pure spring ($E_0$) with $n$ Maxwell arms (i.e., spring $E_i$ in series with a dashpot $\eta_i$) assembled in parallel, thus defining a set of $n$ different characteristic relaxation times $\left(\tau_i=\frac{\eta_i}{E_i}\right)$.[27]

Briefly with $\dot{\varepsilon}$M the viscoelastic parameters are determined following three basic steps: (i) derivation of the transfer function $H(s)$ for a given lumped parameter model in the Laplace domain, (ii) calculation of the response to a fixed strain rate compression ($\dot{\varepsilon}$), and finally (iii) global fitting of experimental stress–time data sets to derive the lumped parameters.

The general form of the GM model transfer function in the Laplace domain can be written as:

$$H_{GM}(s)=\frac{\overline{\sigma}}{\overline{\varepsilon}}=E_0+\sum_{i=1}^{n}\frac{E_i\eta_i s}{E_i+\eta_i s} \tag{A1}$$

where $s$ is the Laplace operator, whereas $\overline{\sigma}$ and $\overline{\varepsilon}$ are, respectively, the stress and the strain in the Laplace domain.

Although any rheological model can be used in general within this framework, only simple Maxwell solid linear standard (i.e., Maxwell SLS) and 2-arm generalized Maxwell (i.e., GM2 model) models were considered here. The transfer functions can be calculated from Eq. (A1) using $n = 1$ for Maxwell SLS [Eq. (A2a)] and $n = 2$ for GM2 model [Eq. (A2b)], obtaining:

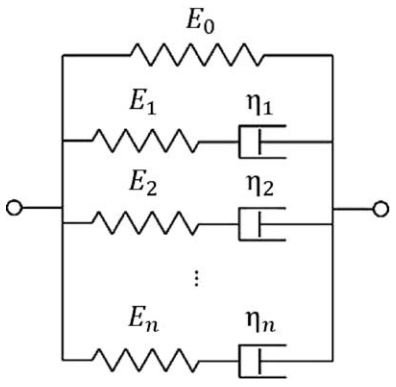


**FIGURE A1**. Generalized Maxwell (GM) model.

$$E_0+\frac{E_1\eta_1 s}{E_1+\eta_1 s} \tag{A2a}$$

and

$$E_0+\frac{E_1\eta_1 s}{E_1+\eta_1 s}+\frac{E_2\eta_2 s}{E_2+\eta_2 s} \tag{A2b}$$

Considering the experimental set-up, in which we have a constant strain rate (represented by $\overline{\varepsilon}s$ in the Laplace domain), Eqs. (A2) can be re-expressed as:

$$\frac{\overline{\sigma}}{\overline{\varepsilon}s}=E_0+\frac{E_1\eta_1}{E_1+\eta_1 s} \tag{A3a}$$

and

$$\frac{\overline{\sigma}}{\overline{\varepsilon}s}=E_0+\frac{E_1\eta_1}{E_1+\eta_1 s}+\frac{E_2\eta_2}{E_2+\eta_2 s} \tag{A3b}$$

In these expressions the constant strain rate input is given by the step function $\frac{\varepsilon_P}{s}$ (in which the amplitude $\varepsilon_P$ is fixed for each test). The model response ($\overline{\sigma}$) to a fixed and constant strain rate compression in the Laplace domain can be easily calculated substituting $\overline{\varepsilon}s$ with $\frac{\varepsilon_P}{s}$ in Eqs. (A3). In case of the models used here, the equations are

$$\overline{\sigma}=\left(\frac{E_0}{s}+\frac{E_1\eta_1}{E_1+\eta_1 s}\right)\cdot\frac{\varepsilon_P}{s} \tag{A4a}$$

and

$$\overline{\sigma}=\left(\frac{E_0}{s}+\frac{E_1\eta_1}{E_1+\eta_1 s}+\frac{E_2\eta_2}{E_2+\eta_2 s}\right)\cdot\frac{\varepsilon_P}{s} \tag{A4b}$$

Finally, the stress–time relations (i.e., $\sigma(t)$) in response to an imposed and constant compressive strain rate are obtained applying an Inverse Laplace transformation, as expressed by the following equation:

$$\sigma(t)=\varepsilon_P\left(\eta_1-\eta_1 e^{-\frac{E_1 t}{\eta_1}}+E_0 t\right) \tag{A5a}$$

and

$$\sigma(t)=\varepsilon_P\left(\eta_1-\eta_1 e^{-\frac{E_1 t}{\eta_1}}+\eta_2-\eta_2 e^{-\frac{E_2 t}{\eta_2}}+E_0 t\right) \tag{A5b}$$

The $\sigma(t)$ equations [reported as Eqs. (4) and (7) in the main text] are finally used in a *global fitting*$\dot{\varepsilon}$ framework to derive lumped parameters.

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