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Viscoelastic Characterisation of Pig Liver in Unconfined Compression

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Abstract

Understanding and modelling liver biomechanics represents a significant challenge due to the complex nature of this organ. Unfortunately, there is no consensus on liver viscoelastic properties, and results are strongly dependent on sample type and status, adopted testing method, and testing conditions. Standard force-triggered tests (e.g. step response or dynamic mechanical tests) necessitate an initial contact between sample and testing apparatus, which may result in significant pre-stress to very soft and highly hydrated samples. In a previous study we proposed the epsilon dot method ($\dot{\varepsilon}M$): a testing and analysis framework to address the drawbacks of standard mechanical tests. Focusing on *ex-vivo* unconfined bulk compressive tests, here we use both $\dot{\varepsilon}M$ and dynamic mechanical analysis (DMA) to derive liver viscoelastic parameters in the region of small strains or the linear viscoelastic region (LVR). As liver samples were visibly deteriorated at the end of frequency sweep tests, a modified approach was adopted to reduce DMA testing times. This approach, termed *step-reconstructed* DMA (SRDMA), is based on dynamic measurements around specific frequencies and then reconstruction of liver behaviour in the entire frequency range of interest. The instantaneous elastic modulus obtained from SRDMA tests (2.65 ± 0.30 kPa) was significantly higher than that obtained with the $\dot{\varepsilon}M$ (2.04 ± 0.01 kPa). We show that the overestimation of stiffness is due to data acquisition in a local rather than an absolute LVR, highlighting the importance of using a rapid and zero pre-stress approach to characterise very soft and highly hydrated biological tissues.

1. Introduction

Although the mechanical properties of structural materials have been well described for decades using various testing methods, there is still a scarcity of reliable and reproducible data for most biological soft tissues. Characterising the heterogeneous, non-linear viscoelastic behaviour of soft non load-bearing tissues (such as liver, kidney and brain) is very challenging, and a standard testing method is needed to produce repeatable results that can be mathematically modelled to derive the mechanical behaviour of a given tissue. Ideally, tissue characterisation via constitutive modelling requires the control of both geometric and environmental boundary conditions. One of the main difficulties in developing appropriate viscoelastic models for soft tissues is the establishment of suitable experimental testing setups and protocols for the unique identification of material parameters. Generally, soft tissues can be characterised under two different conditions, namely *in-vivo* and *ex-vivo*. *In-vivo* testing maintains the tissue in its natural state, but has many limitations, such as accessibility, ill-defined boundary conditions, ethical issues in using animals and potential risks to human subjects. Several *in-vivo* mechanical measurements are reported in the literature, with datasets often limited to small deformations. Furthermore, the interpretation of data is challenging due to difficulties in obtaining appropriate alignment between the instrument and tested specimens, the presence of physiological noise and the inability to account for and control the internal condition of the organ (Brown et al., 2003; Gefen and Margulies, 2004; Tay et al., 2002). Conversely, *ex-vivo* experiments are preferable when developing testing devices and protocols, as well for ease of testing, control of boundary conditions and ethical considerations (Gao et al., 2010; Ocal et al., 2010; Raghunathan et al., 2010; Sakuma et al., 2003; Valtorta and Mazza, 2005). They are also suitable for the development of mechanically matched biomimetic scaffolds for in-vitro models and tissue engineering.

Like most internal organs, the liver essentially consists of a functional highly vascularised core composed of cells embedded in a hydrated, porous and intrinsically viscoelastic extracellular matrix (ECM). The organ is covered with a connective tissue capsule (i.e. the Glisson's capsule) made of densely interwoven collagen fibres, which ensures its structural integrity (Brunon et al., 2010). Several methods and models are reported in the literature to characterise the mechanical behaviour of liver either *ex-vivo* or *in-vivo* based on: direct measurements on tissue (e.g. rheological (Kalanovic et al., 2003; Liu and Bilston, 2000; Marchesseau et al., 2010), compressive (Gao et al., 2010; Kemper et al., 2013; Pervin et al., 2011; Raghunathan et al., 2010), indentation tests (Jordan et al., 2009; Kerdok et al., 2006)) or imaging techniques (e.g. magnetic resonance (Asbach et al., 2008; Clarke et al., 2011; Haghpanahi and Naeeni, 2010; Klatt et al., 2007; Venkatesh et al., 2008), ultrasound-based elastography (Adebajo et al., 2012; Chenot et al., 2009; Ferraioli et al., 2013; Yoon et al., 2012)). The first studies on the mechanical behaviour of animal and human livers were principally focused on the investigation of static material properties (Carter et al., 2001; Nava et al., 2008; Roan and Vemaganti, 2007; Tay et al., 2002). Viscoelastic properties have been explored only recently, typically using stress-relaxation and dynamic loading experiments to measure either time- or frequency-dependent material properties, respectively (Liu and Bilston, 2000; Pervin et al., 2011). The development of imaging elastography systems has also contributed to the study of the frequency dependence of liver mechanical properties (Kruse et al., 2000; Valtorta and Mazza, 2005). Chatelin et al. compared *in-vivo* ultrasound-based transient elastography (TE) and *ex-vivo* rheometry tests (DMA) on porcine livers, demonstrating that the elastic properties measured by the two methods are equivalent (Chatelin et al., 2011). It is worth noting that due to its highly heterogeneous structure, most ~~of the~~ mechanical models of liver published to date are not material constitutive laws, but rather a means to quantify the gross tissue mechanical properties and to determine the time scales of liver viscoelasticity, often attempting to

correlate them with different tissue conditions such as pathophysiological states or tissue ageing. Indeed, despite the large number of investigations and reports, there is no consensus on the mechanical properties of liver, or other soft tissues in general. Reported dynamic moduli span from few to tens of kPa (Marchesseau et al., 2010) and are strongly dependent on the adopted testing method and experimental conditions as well as sample type. In particular, the physical condition of the tissue (Kerdok et al., 2006), post-mortem time or preservation period (Ocal et al., 2010), pathophysiological state (Mazza et al., 2007; Wang et al., 1992; Yeh et al., 2002), tissue preload (Clarke et al., 2011; Yeh et al., 2002) and gravity (Gao et al., 2010) can all affect sample status, and hence its mechanical properties. Moreover the specific tissue model used, e.g. purely elastic versus viscoelastic, strongly conditions the estimated tissue viscoelastic parameters.

Clearly, this is a vast and multifaceted area of research wherefrom emerges the need to clearly identify the parameters of interest and then, based on available testing equipment, choose the appropriate experimental set-up and analysis method. This need, coupled with the high variability and inconsistency of published data on liver mechanical properties, motivated us to develop a reliable quantitative testing and analysis framework for characterising the viscoelastic mechanical behaviour of very soft and highly hydrated biological tissues *ex-vivo.* In this context, our main focus was the use of common mechanical testing apparatus to measure hepatic tissue properties in the region of small and physiologically relevant deformations, where soft tissues can be approximated as linear viscoelastic materials. In general, soft tissue mechanical testing is beset by two main problems. First, the issues related to establishing working conditions which ensure that each sample is in the same reproducible status before testing. Secondly, standard force- or strain-triggered tests (e.g. step response or dynamic mechanical tests) are affected by the long duration of tests and the need of an initial contact between sample and testing apparatus, likely causing significant pre-stress and sample deterioration. Our aim

was thus to standardise sample preparation with defined, controlled and rapid testing conditions in order to minimise tissue deterioration and guarantee a standard reproducible initial sample status. Hence, two different testing methods were established to derive the viscoelastic parameters of hepatic tissue through unconfined compressive tests within the linear viscoelastic region (LVR).

The first is based on the $\dot{\varepsilon}M$ (epsilon dot method) which we recently proposed for testing and parameter derivation of soft hydrated materials (Tirella et al., 2013), while the second is the dynamic mechanical analysis (DMA) with a restricted number of discrete frequencies to reduce the duration of the test (i.e. *step-reconstructed* DMA, or SRDMA).

With both methods, viscoelastic parameters of the hepatic tissue were estimated using a global fitting approach with shared parameters. The SRDMA and $\dot{\varepsilon}M$ are discussed and compared, highlighting the similarities, advantages and limitations of the two methods for characterising the viscoelastic behaviour of very soft, degradable and highly hydrated biological materials such as hepatic tissue.

2. Materials and methods

2.1. Sample preparation

Fresh porcine livers from 1 year old healthy pigs were collected as a slaughter by-product and frozen at -20 °C within 3 hours of death. Prior to use, frozen livers were thawed at 4 °C overnight, then punched to obtain regular 14 mm diameter cylinders which were subsequently cut in 3 mm thick samples with parallel loading surfaces using a custom slicer and a microtome blade. Capsular connective tissue (i.e. Glisson's capsule) was not present in tested samples and particular attention was dedicated to avoid macroscopic vasculature. We recently showed that the bulk compressive modulus (λ) of porcine fresh liver does not change significantly with the sample harvesting site (i.e. different liver lobes) nor

between animals from the same slaughterhouse (Mattei et al., 2014). Furthermore, in agreement with Tamura et al. (Tamura et al., 2002), our results showed that a freeze-thawing cycle (samples stored at -20 °C, then thawed at 4 °C overnight prior to testing) does not significantly affect the liver compressive modulus. Hence, thawed samples from multiple harvesting sites were used for all tests. To ensure repeatable testing conditions, thawed liver samples were equilibrium swollen in PBS 1X at 4 °C and then brought to room temperature prior to testing (Mattei et al., 2014; Yeh et al., 2002). Samples were carefully measured in thickness (l_0) and diameter (d) just before testing (hence accounting for any size variations due the swelling process). Measurements were performed by gently placing the jaws of a calliper (0.05 mm resolution) in contact with the sides of the sample, averaging readings from at least three different points. Samples were tested partially immersed in PBS 1X to preserve their hydration during the unconfined compression test (Mattei et al., 2014; Tirella et al., 2013). Mechanical tests were performed within 2 weeks after sample collection.

2.2. $\dot{\varepsilon}M$

The $\dot{\varepsilon}M$ is based on the application of a series of short compressions at different strain rates to specimens while acquiring force and displacement versus time data in the LVR (Tirella et al., 2013). Tests were performed with a uniaxial testing device (Zwick/Roell ProLine Z005) equipped with a 10 N load cell (Zwick/Roell Xforce HP 10 N), applying strain rates of 0.001, 0.005, 0.01 and 0.05 s^{-1} to liver samples. In particular, force and displacement versus time data were acquired starting with the upper plate of the testing device (connected to the load cell) close to but not in contact with the sample, to guarantee a zero pre-stress initial condition and a constant approach velocity. Experimental force- and displacement-time series were respectively normalised to sample cross-sectional area ($\pi d^2/4$) and

thickness (l_0) measured just prior to testing, obtaining stress- and strain-time series. Liver LVR was identified as the region in which stress varies linearly with applied strain (R^2 of at least 0.995). Then, stress-time data within LVR obtained from measurements at different strain rates were used to derive viscoelastic constants for lumped parameter models using the global fitting procedure with shared parameters described in Supplementary Information (SI 3). Six liver samples were tested at each strain rate, using a new sample for each repeat; total number of specimens = 24.

2.3. Dynamic mechanical testing method

Dynamic mechanical analysis (DMA), a standard force-triggered method, was used to determine material viscoelastic properties by applying a small amplitude cyclic strain on a sample and measuring the resultant cyclic stress response. The tests were performed compressing samples at room temperature using a GABO Eplexor 150 N (Gabo GmbH, Ahlden, Germany). The trigger force was set to a value of 10 mN, which we identified as the minimum reliable starting force of the instrument. In this set of experiments, the LVR was considered as the range of strain amplitudes in which the storage modulus changes by less than 5% of its initial value. To identify the LVR for frequency sweep tests, a preliminary series of strain amplitude sweep tests at 1 Hz was conducted.

2.3.1. Conventional dynamic mechanical tests

To assess any possible change in liver mechanical properties due to tissue deterioration while standing, equilibrium swollen samples were kept in PBS 1X at room temperature for different times prior to testing (i.e. 0, 2, 4, 20, 24 h). Frequency sweep tests were then performed in triplicate in the frequency range 0.05÷100 Hz, choosing 1% static and 0.5% dynamic strain amplitudes to guarantee a linear

response, as outlined in Supplementary Information SI 1. Note that each sweep test took about 1 hour and 30 minutes to complete.

2.3.2. *Step-reconstructed* dynamic mechanical tests

The SRDMA approach is based on performing short frequency sweeps around selected frequencies (f = 0.5, 1, 10 and 50 Hz): specifically, measurements were performed at the selected frequency f, and $f \pm$ 0.1 Hz on the same sample. Equilibrium swollen samples were tested after being brought to room temperature choosing 1% static and 0.5% dynamic strain amplitudes to lie within liver LVR. Liver dynamic mechanical behaviour was then reconstructed over the whole investigated frequency-range (SRDMA) as described in the Supplementary Information. Compared with the 0.05 ÷ 100 Hz frequency sweep test, this approach enables a shorter testing phase (i.e. less than 5 minutes in the longest test, 0.4 ÷ 0.6 Hz frequency range), hence preventing any significant sample deterioration. Note that, since each sample was tested only once to prevent permanent alterations due to repeated testing cycles, 12 liver samples were required to perform the SRDMA analysis in triplicate around the four selected frequencies. Cyclic tests at each of the single frequencies investigated around 1 Hz were also performed on independent samples. No significant differences in E' and E'' were measured between independent samples tested at 0.9, 1 and 1.1 Hz with respect to those obtained by sequential tests at 0.9, 1 and 1.1 Hz on the same sample (data not reported), confirming the absence of sub-failure loading in the SRDMA approach.

2.4. Lumped parameter estimation

Liver samples were treated as mechanically isotropic materials (Marchesseau et al., 2010; Mattei et al., 2014; Pervin et al., 2011). In the region of small deformations, the viscoelastic behaviour of soft and

hydrated materials can be derived using classical lumped parameter models such as the Maxwell Standard Linear Solid (SLS) (SI 1). As described in the Supplementary Information, two models, the SLS and the GM2 (2-arm Generalised Maxwell model) were used to estimate the material coefficients for liver tissue (SI 2). A global fitting approach was employed performing *chi-square* minimisation in a combined parameter space (SI 3). In order to select suitable parameter initial guesses, an annealing scheme, multiplying and dividing each initial parameter by 10 individually while keeping the instantaneous modulus at a constant value (i.e. a constant sum of all springs in the model), was adopted. In this way reliable and absolute hepatic viscoelastic parameters within the investigated frequency range were obtained, while avoiding most of the local minima during the fitting procedure. A lower boundary was set to prevent the fitting procedure returning negative values for the estimated viscoelastic coefficients. Comparisons between parameter values were made using the Student's t-test, setting significance at $p < 0.05$.

3. Results

3.1. $\dot{\varepsilon}M$

As sketched in Fig. 1a, the measured sample stress is zero while the plate approaches the sample, prior to the instant of contact to ensure no pre-stress acting on tested sample (zone A). Further advancement results in a slight negative stress, mainly due to water mediated adhesive forces between the plate and the sample (zone B). Zone C, represents the actual compression of the sample: in this region the stress increases monotonically with time. Experimental stress-time data collected at various strain rates are shown in Fig. 1b, where the time axis has been offset to be zero at the beginning of sample compression (i.e. zone C). Only stress-time data belonging to the LVR are shown. In all experiments the LVR extended up to a strain of 0.03, therefore the higher the strain-rate, the shorter the duration of

the stress-time series (Fig. 1b). Liver samples did not demonstrate any visible changes at the end of the rapid $\dot{\varepsilon}M$ compressive tests.

3.2. DMA: frequency sweep test

Using the conventional DMA approach, strain amplitude sweep tests underlined that sample LVR extended up to 2% strain, in accordance with Marchesseau et al. (Marchesseau et al., 2010). However, at the end of the frequency sweep test, samples were found to be highly compressed, dark brown and dehydrated likely due to the very long testing time. This is clearly highlighted in Fig. 2 in which the sample height decreases rapidly at the lower frequencies. As the sweep begins, cyclic stresses cause fluid expulsion from the tested sample, so that additional compression is required to reach the trigger contact force necessary for successive testing cycles.

Consequently, the measured storage and loss moduli likely reflect sample deterioration and water elimination rather than the dynamic mechanical properties. In fact, no meaningful trends in E' and E'' were found between measurements performed at different preservation times (Fig. 3) and the experimental results were not used further to derive liver viscoelastic parameters.

3.3. SRDMA

We did not observe any deterioration in samples using the quicker SRDMA approach and sample compression at the end of the test was measured to be less than 2% of its initial height.

SRDMA storage and loss moduli are presented in Fig. 4. As expected, the storage modulus increases with frequency up to the relaxation frequency and then remains almost constant. On the other hand, the

loss modulus increases with frequency reaching a peak value at the relaxation frequency (~ 1 Hz, at which maximum energy dissipation occurs) and then decreases to zero as the frequency is further increased, showing that liver mechanical behaviour is mainly elastic at high frequencies.

3.4. Lumped parameter estimation using global fitting

Fitting results for Maxwell SLS model are summarised in Table 1 where E_{inst} and E_{eq} represent the instantaneous (i.e. sum of all springs in the model) and equilibrium (i.e. E_0) moduli, respectively, while τ_i is the i^{th} -arm characteristic relaxation time, calculated as η_i/E_i. Although convergence was obtained for both SRDMA and the $\dot{\varepsilon}M$, fitting the datasets to the GM2 model yielded non-significant results (data reported in Supplementary Information, SI 4) with very large standard errors, clearly indicating model over-parameterization for both $\dot{\varepsilon}M$ and SRDMA.

4. Discussion

Given that most testing methods for viscoelastic characterisation are overly long and may therefore deteriorate labile or living tissues, we investigated two different methods for rapid testing of soft materials in unconfined compression. Focusing on small deformations in the liver, all samples were tested in the LVR. The $\dot{\varepsilon}M$ consists in a short series of constant strain rate tests to determine lumped parameters from a series of stress-time data, while the SRDMA is based on a short series of frequency sweeps centred around selected frequencies of interest. The duration of the testing phase depends on the strain rate for $\dot{\varepsilon}M$ or on the frequency employed for SRDMA: the lower the $\dot{\varepsilon}$ or f the longer the test. In case of $\dot{\varepsilon}M$ we used strain rates between 0.001 and 0.05 s^{-1}, while for SRDMA a frequency

range of 0.5 to 50 Hz was employed, thus tests were shorter than 30 s for the former and 300 s for the latter. In terms of magnitude, SRDMA storage and loss moduli are comparable to those reported by Liu and Bilston for bovine liver (G' = 1 – 6 kPa, G'' < 1 kPa, f = 0.006 – 20 Hz) (Liu and Bilston, 2000), Kiss et al. for canine liver (E' = 3 – 8 kPa, E'' = 0.8 – 4 kPa, f = 0.1 – 100 Hz) (Kiss et al., 2004) and Marchesseau et al. for porcine liver (G' = 0.3 – 0.6 kPa, G'' = 0.05 – 0.15 kPa, f = 0.1 – 4 Hz) (Marchesseau et al., 2010). However, the estimated viscoelastic parameters of liver, or highly non-linear soft materials in general, are strongly dependent on the testing conditions.

As mentioned in Section 2.3, a minimum contact force of 10 mN is necessary to trigger the GABO Eplexor 150 N. Given that sample surface area of samples is about 1.5 cm^2, the trigger force results in an average sample pre-stress of 65 Pa with a consequent strain of about 4%. In this region, a local LVR can nevertheless be identified (Figure 5), albeit with a smaller linear range and a higher slope with respect to that obtained in the absence of pre-stress (i.e. the absolute LVR).

To perform measurements in the absolute LVR, it is necessary to use methods which do not require a trigger force during the acquisition phase. In this perspective, the $\dot{\varepsilon}M$ is very suited for the testing of soft materials, as viscoelastic parameters can be derived with standard uniaxial testing devices, starting in a contactless configuration (Tirella et al., 2013). In fact without pre-stress, E_{inst} was found to be significantly lower (2.04 ± 0.01 kPa) than the value estimated with SRDMA (2.65 ± 0.30 kPa, p = 0.0174), demonstrating that the unavoidable trigger contact force causes sample stiffening. The effect of the trigger force is also reflected in the characteristic relaxation time: as shown in Table 1, the value of τ estimated with the SRDMA was significantly lower than that obtained using the $\dot{\varepsilon}M$ (p < 0.0001), suggesting that pre-stressed samples exhibit a more elastic behaviour than in the absence of pre-stress.

Clearly, the estimated time constants depend strongly on the range of strain rates (in the case of $\dot{\varepsilon}M$) or frequencies (in the case of SRDMA) employed during testing. In both cases, an *a priori* knowledge of the relaxation behaviour of a material is desirable if one wishes to capture material viscoelastic features within a time frame of interest.

5. Conclusion

The objective of this study was to formulate a reliable and reproducible method for the rapid testing and measurement of liver viscoelastic properties in the true LVR. To this end, two different methods (SRDMA and $\dot{\varepsilon}M$) were developed and used to derive the viscoelastic parameters of porcine liver samples. Attention was paid to tissue handling and treatment throughout the study, ensuring all samples were in a repeatable initial state. The study highlights the advantages and disadvantages of SRDMA and $\dot{\varepsilon}M$, in testing soft and degradable biomaterials. Although both methods permit considerable time savings and good sample preservation, in our opinion the $\dot{\varepsilon}M$ can give a better estimation of the viscoelastic parameters than does SRDMA, since it avoids sample pre-stress and allows measurements in the actual LVR. The $\dot{\varepsilon}M$ is, as far as we know, the only testing and analysis framework which enables the unique identification of viscoelastic parameters of soft materials - such as hepatic tissue - through direct measurements and in the absence of pre-stress. The results can be used to design mechanically matched hepatic tissue models and can also be extended to the characterisation and constitutive modelling of other soft tissues and materials, enabling comparisons across different studies.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Adebajo, C.O., Talwalkar, J.A., Poterucha, J.J., Kim, W.R., Charlton, M.R., 2012. Ultrasound-based transient elastography for the detection of hepatic fibrosis in patients with recurrent hepatitis C virus after liver transplantation: a systematic review and meta-analysis. Liver Transpl. 18, 323–31.

Asbach, P., Klatt, D., Hamhaber, U., Braun, J., Somasundaram, R., Hamm, B., Sack, I., 2008. Assessment of liver viscoelasticity using multifrequency MR elastography. Magn. Reson. Med. 60, 373–9.

Brown, J.D., Rosen, J., Kim, Y.S., Chang, L., Sinanan, M.N., Hannaford, B., 2003. In-vivo and in-situ compressive properties of porcine abdominal soft tissues. Stud. Health Technol. Inform. 94, 26–32.

Brunon, A., Bruyère-Garnier, K., Coret, M., 2010. Mechanical characterization of liver capsule through uniaxial quasi-static tensile tests until failure. J. Biomech. 43, 2221–7.

Carter, F.J., Frank, T.G., Davies, P.J., McLean, D., Cuschieri, A., 2001. Measurements and modelling of the compliance of human and porcine organs. Med. Image Anal. 5, 231–6.

Chatelin, S., Oudry, J., Périchon, N., Sandrin, L., Allemann, P., Soler, L., Willinger, R., 2011. In vivo liver tissue mechanical properties by Transient Elastography: comparison with Dynamic Mechanical Analysis. Biorheology 48, 75–88.

Chenot, J., Melodelima, D., Parmentier, H., Souchon, R., Chapelon, J.-Y., 2009. Comparison of different compression methods for ultrasound elastography in order to detect HIFU ablations in

the liver: preliminary in vivo results. In: 2009 IEEE International Ultrasonics Symposium. IEEE, pp. 135–138.

Clarke, E.C., Cheng, S., Green, M., Sinkus, R., Bilston, L.E., 2011. Using static preload with magnetic resonance elastography to estimate large strain viscoelastic properties of bovine liver. J. Biomech. 44, 2461–5.

Ferraioli, G., Tinelli, C., Dal Bello, B., Zicchetti, M., Lissandrin, R., Filice, G., Filice, C., Above, E., Barbarini, G., Brunetti, E., Calderon, W., Di Gregorio, M., Gulminetti, R., Lanzarini, P., Ludovisi, S., Maiocchi, L., Malfitano, A., Michelone, G., Minoli, L., Mondelli, M., Novati, S., Patruno, S.F.A., Perretti, A., Poma, G., Sacchi, P., Zanaboni, D., Zaramella, M., 2013. Performance of liver stiffness measurements by transient elastography in chronic hepatitis. World J. Gastroenterol. 19, 49–56.

Gao, Z., Lister, K., Desai, J.P., 2010. Constitutive modeling of liver tissue: experiment and theory. Ann. Biomed. Eng. 38, 505–16.

Gefen, A., Margulies, S.S., 2004. Are in vivo and in situ brain tissues mechanically similar? J. Biomech. 37, 1339–52.

Haghpanahi, M., Naeeni, H.A., 2010. Investigation of viscoelastic properties of human liver tissue using MR elastography and FE modeling. In: 2010 17th Iranian Conference of Biomedical Engineering (ICBME). IEEE, pp. 1–4.

Jordan, P., Socrate, S., Zickler, T.E., Howe, R.D., 2009. Constitutive modeling of porcine liver in indentation using 3D ultrasound imaging. J. Mech. Behav. Biomed. Mater. 2, 192–201.

Kalanovic, D., Ottensmeyer, M.P., Gross, J., Buess, G., Dawson, S.L., 2003. Independent testing of soft tissue visco-elasticity using indentation and rotary shear deformations. Stud. Health Technol. Inform. 94, 137–43.

Kemper, A.R., Santago, A.C., Stitzel, J.D., Sparks, J.L., Duma, S.M., 2013. Effect of strain rate on the material properties of human liver parenchyma in unconfined compression. J. Biomech. Eng. 135, 104503–8.

Kerdok, A.E., Ottensmeyer, M.P., Howe, R.D., 2006. Effects of perfusion on the viscoelastic characteristics of liver. J. Biomech. 39, 2221–31.

Kiss, M.Z., Varghese, T., Hall, T.J., 2004. Viscoelastic characterization of in vitro canine tissue. Phys. Med. Biol. 49, 4207–18.

Klatt, D., Hamhaber, U., Asbach, P., Braun, J., Sack, I., 2007. Noninvasive assessment of the rheological behavior of human organs using multifrequency MR elastography: a study of brain and liver viscoelasticity. Phys. Med. Biol. 52, 7281–94.

Kruse, S.A., Smith, J.A., Lawrence, A.J., Dresner, M.A., Manduca, A., Greenleaf, J.F., Ehman, R.L., 2000. Tissue characterization using magnetic resonance elastography: preliminary results. Phys. Med. Biol. 45, 1579–90.

Liu, Z., Bilston, L., 2000. On the viscoelastic character of liver tissue: experiments and modelling of the linear behaviour. Biorheology 37, 191–201.

Marchesseau, S., Heimann, T., Chatelin, S., Willinger, R., Delingette, H., 2010. Fast porous visco-hyperelastic soft tissue model for surgery simulation: application to liver surgery. Prog. Biophys. Mol. Biol. 103, 185–96.

Mattei, G., Di Patria, V., Tirella, A., Alaimo, A., Elia, G., Corti, A., Paolicchi, A., Ahluwalia, A., 2014. Mechanostructure and composition of highly reproducible decellularized liver matrices. Acta Biomater. 10, 875–82.

Mazza, E., Nava, A., Hahnloser, D., Jochum, W., Bajka, M., 2007. The mechanical response of human liver and its relation to histology: an in vivo study. Med. Image Anal. 11, 663–72.

Nava, A., Mazza, E., Furrer, M., Villiger, P., Reinhart, W.H., 2008. In vivo mechanical characterization of human liver. Med. Image Anal. 12, 203–16.

Ocal, S., Ozcan, M.U., Basdogan, I., Basdogan, C., 2010. Effect of preservation period on the viscoelastic material properties of soft tissues with implications for liver transplantation. J. Biomech. Eng. 132, 101007.

Pervin, F., Chen, W.W., Weerasooriya, T., 2011. Dynamic compressive response of bovine liver tissues. J. Mech. Behav. Biomed. Mater. 4, 76–84.

Raghunathan, S., Evans, D., Sparks, J.L., 2010. Poroviscoelastic modeling of liver biomechanical response in unconfined compression. Ann. Biomed. Eng. 38, 1789–800.

Roan, E., Vemaganti, K., 2007. The nonlinear material properties of liver tissue determined from no-slip uniaxial compression experiments. J. Biomech. Eng. 129, 450–6.

Sakuma, I., Nishimura, Y., Chui, C.K., Kobayashi, E., 2003. In vitro Measurement of Mechanical Properties of Liver Tissue under Compression and Elongation Using a New Test Piece Holding Method with Surgical Glue, In Vitro, Lecture Notes in Computer Science. Springer Berlin Heidelberg, Berlin, Heidelberg.

Tamura, A., Omori, K., Miki, K., Lee, J.B., Yang, K.H., King, A.I., 2002. Mechanical characterization of porcine abdominal organs. Stapp Car Crash J. 46, 55–69.

Tay, B.K., Stylopoulos, N., De, S., Rattner, D.W., Srinivasan, M.A., 2002. Measurement of in-vivo force response of intra-abdominal soft tissues for surgical simulation. Stud. Health Technol. Inform. 85, 514–9.

Tirella, A., Mattei, G., Ahluwalia, A., 2013. Strain rate viscoelastic analysis of soft and highly hydrated biomaterials. J. Biomed. Mater. Res. A.

Valtorta, D., Mazza, E., 2005. Dynamic measurement of soft tissue viscoelastic properties with a torsional resonator device. Med. Image Anal. 9, 481–90.

Venkatesh, S.K., Yin, M., Glockner, J.F., Takahashi, N., Araoz, P.A., Talwalkar, J.A., Ehman, R.L., 2008. MR elastography of liver tumors: preliminary results. AJR. Am. J. Roentgenol. 190, 1534–40.

Wang, B.C., Wang, G.R., Yan, D.H., Liu, Y.P., 1992. An experimental study on biomechanical properties of hepatic tissue using a new measuring method. Biomed. Mater. Eng. 2, 133–8.

Yeh, W.-C., Li, P.-C., Jeng, Y.-M., Hsu, H.-C., Kuo, P.-L., Li, M.-L., Yang, P.-M., Lee, P.H., 2002. Elastic modulus measurements of human liver and correlation with pathology. Ultrasound Med. Biol. 28, 467–474.

Yoon, K.T., Lim, S.M., Park, J.Y., Kim, D.Y., Ahn, S.H., Han, K.-H., Chon, C.Y., Cho, M., Lee, J.W., Kim, S.U., 2012. Liver stiffness measurement using acoustic radiation force impulse (ARFI) elastography and effect of necroinflammation. Dig. Dis. Sci. 57, 1682–91.

Figure Legends

Fig. 1: Epsilon dot method. a) Schematic of experimental testing setup and of a typical stress-time curve recorded during an $\dot{\varepsilon}M$ test. The measured stress is zero while the plate approaches the sample (zone A). Then it becomes slightly negative due to water mediated adhesive forces between the plate and the sample (zone B). Finally, the measured stress becomes positive, increasing monotonically with time, defining the zone of actual sample compression (zone C). b) Experimental stress-time data collected at various strain rates. The time axis has been offset to be zero at the beginning of the actual compressing phase (zone C) and only stress-time data belonging to the LVR (i.e. those used to estimate lumped parameters) are shown

Fig. 2 Change in sample height during frequency sweep measurements reflecting sample degradation while testing, $n = 3$

Fig. 3 Experimental a) E' and b) E'' of liver samples tested at different preservation times

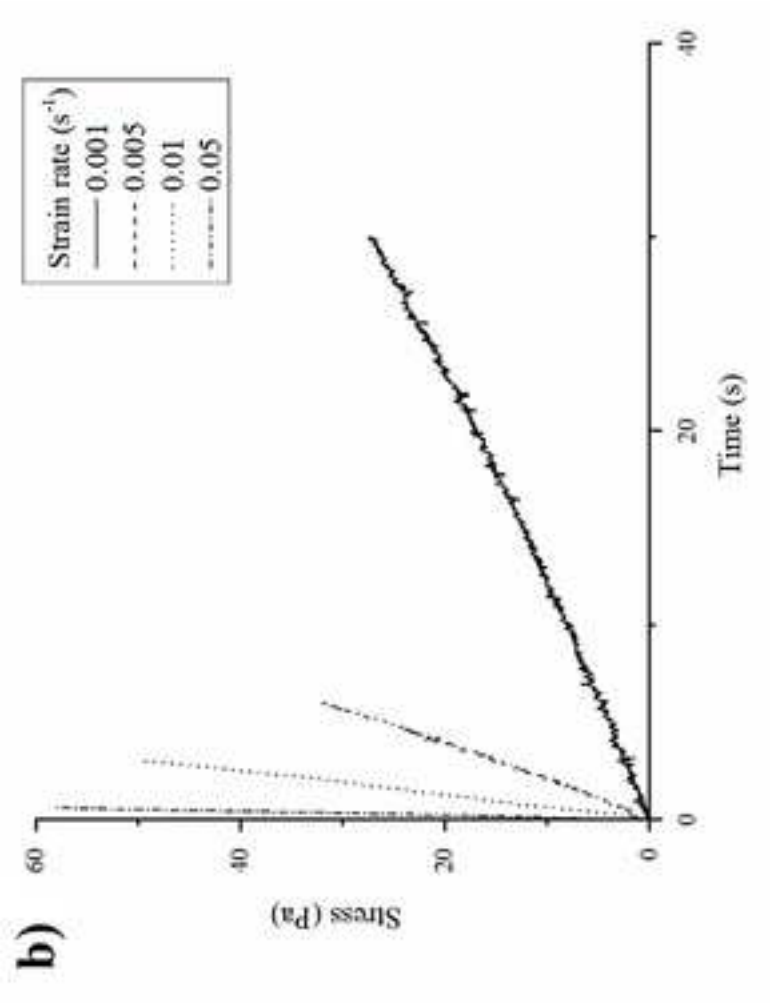
Fig. 4 Experimental a) E' and b) E'' obtained for liver tissue with SRDMA. Measurements were carried out at 12 frequencies ($f = 0.5 \pm 0.1$, 1 ± 0.1, 10 ± 0.1 and 50 ± 0.1 Hz). Each point represents an average value of $n = 3$ independent experiments, while the dashed curve shows the SLS fitting

Fig. 5 Liver stress-strain plot up to 0.10 true compressive strain, data obtained from an $\dot{\varepsilon}M$ test at 0.01 s^{-1}. Clearly, the absolute liver LVR is comprised in the 0 – 0.03 strain range, while local LVRs can be found depending on the measurement's starting point (i.e. pre-strain). SRDMA analysis suffers from a 4% pre-strain due to GABO Eplexor's triggering force, from which a local LVR can be found, characterised by a smaller linear range and a higher slope with respect to the absolute LVR obtained in the absence of pre-stress, as outlined in the table insert

Table Legends

Tab. 1 Viscoelastic parameters of porcine liver estimated for Maxwell SLS lumped model using $\dot{\varepsilon}M$ and SRDMA. Results are expressed as estimated parameter value ± standard error

	$\dot{\varepsilon}M$	SRDMA
E_{inst} (kPa)	2.04 ± 0.01	2.65 ± 0.30
E_{eq} (kPa)	0.91 ± 0.01	0.89 ± 0.22
τ_1 (s)	1.10 ± 0.02	0.20 ± 0.06
R^2	0.97	0.92


b)
Stress (Pa)
Time (s)
Strain rate (s-1)
0.001
0.005
0.01
0.05
0
20
40
60

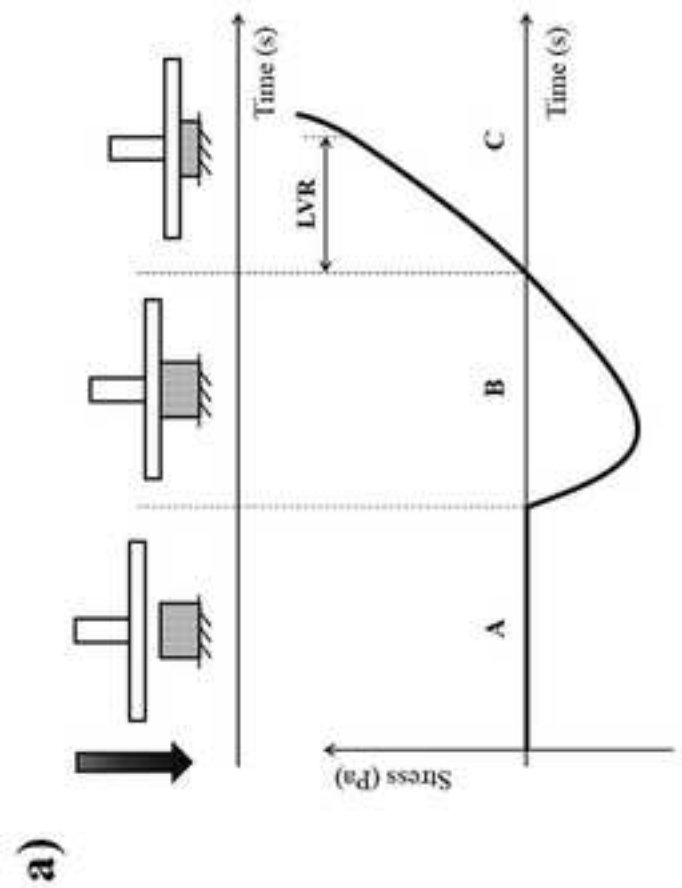

a)
Stress (Pa)
Time (s)
Time (s)
LVR
A
B
C

Figure 1

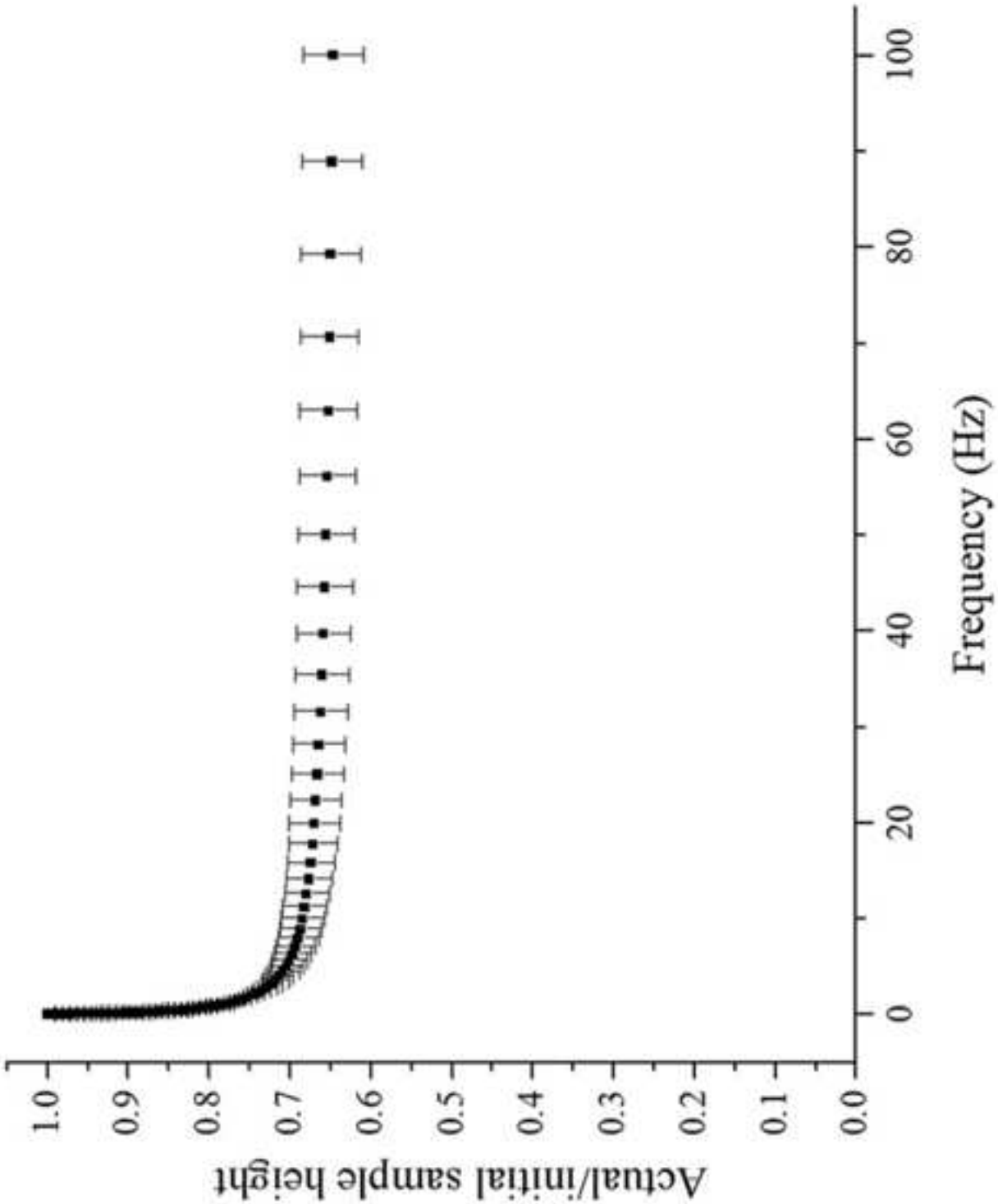


Figure 2

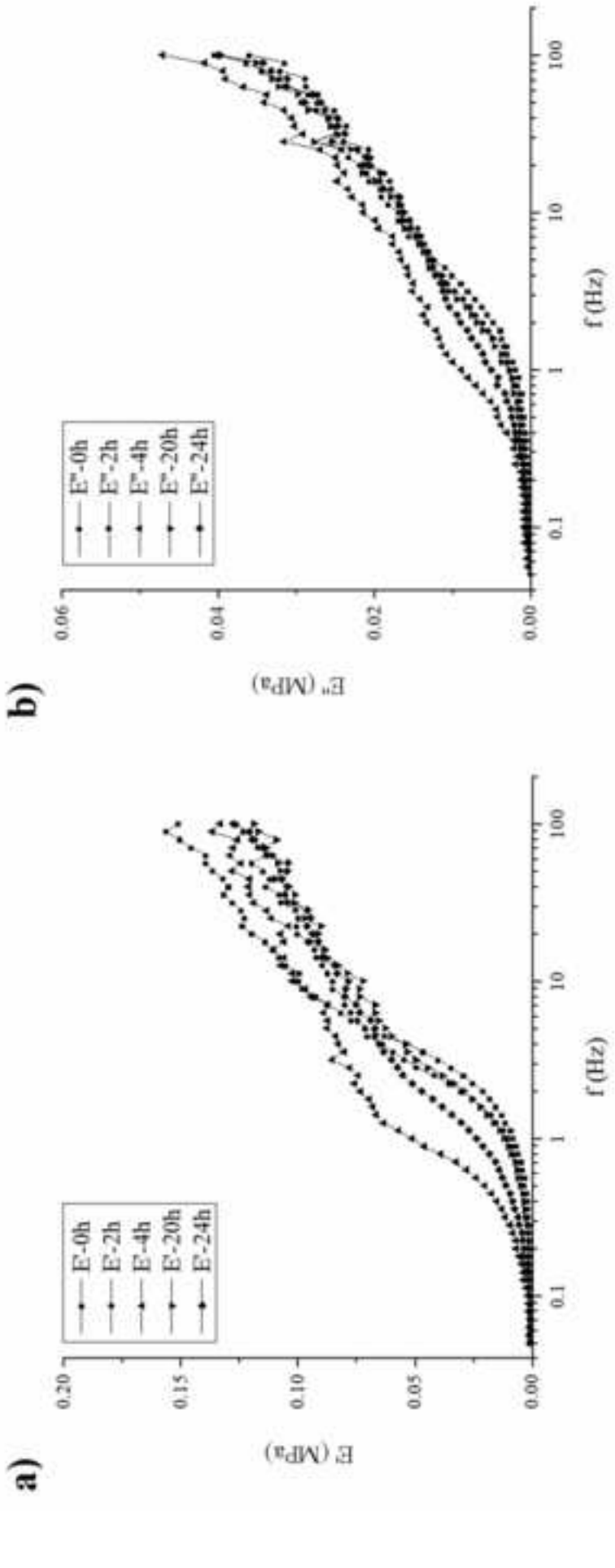

a)
E (MPa)
0.20
0.15
0.10
0.05
0.00
E'-0h
E'-2h
E'-4h
E'-20h
E'-24h
0.1
1
10
100
f (Hz)
b)
E" (MPa)
0.06
0.04
0.02
0.00
E"-0h
E"-2h
E"-4h
E"-20h
E"-24h
0.1
1
10
100
f (Hz)

Figure 3

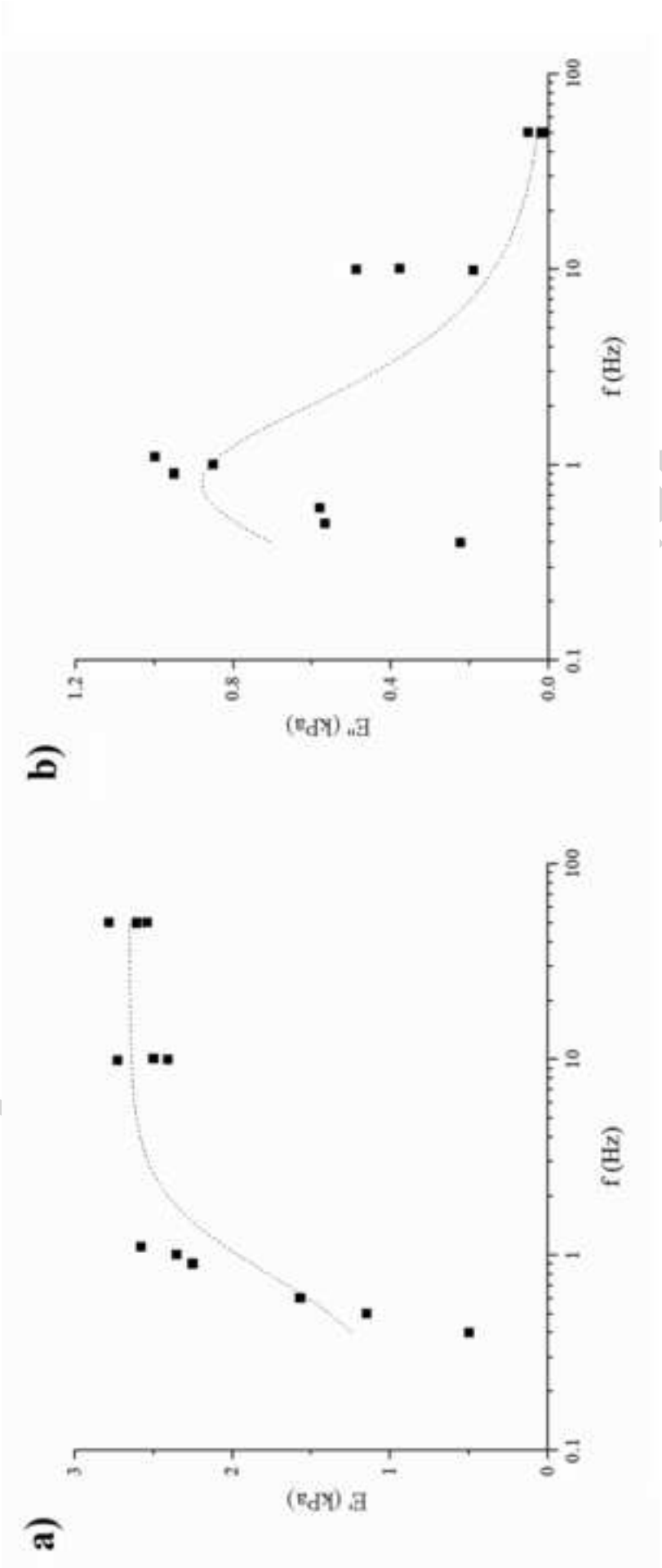
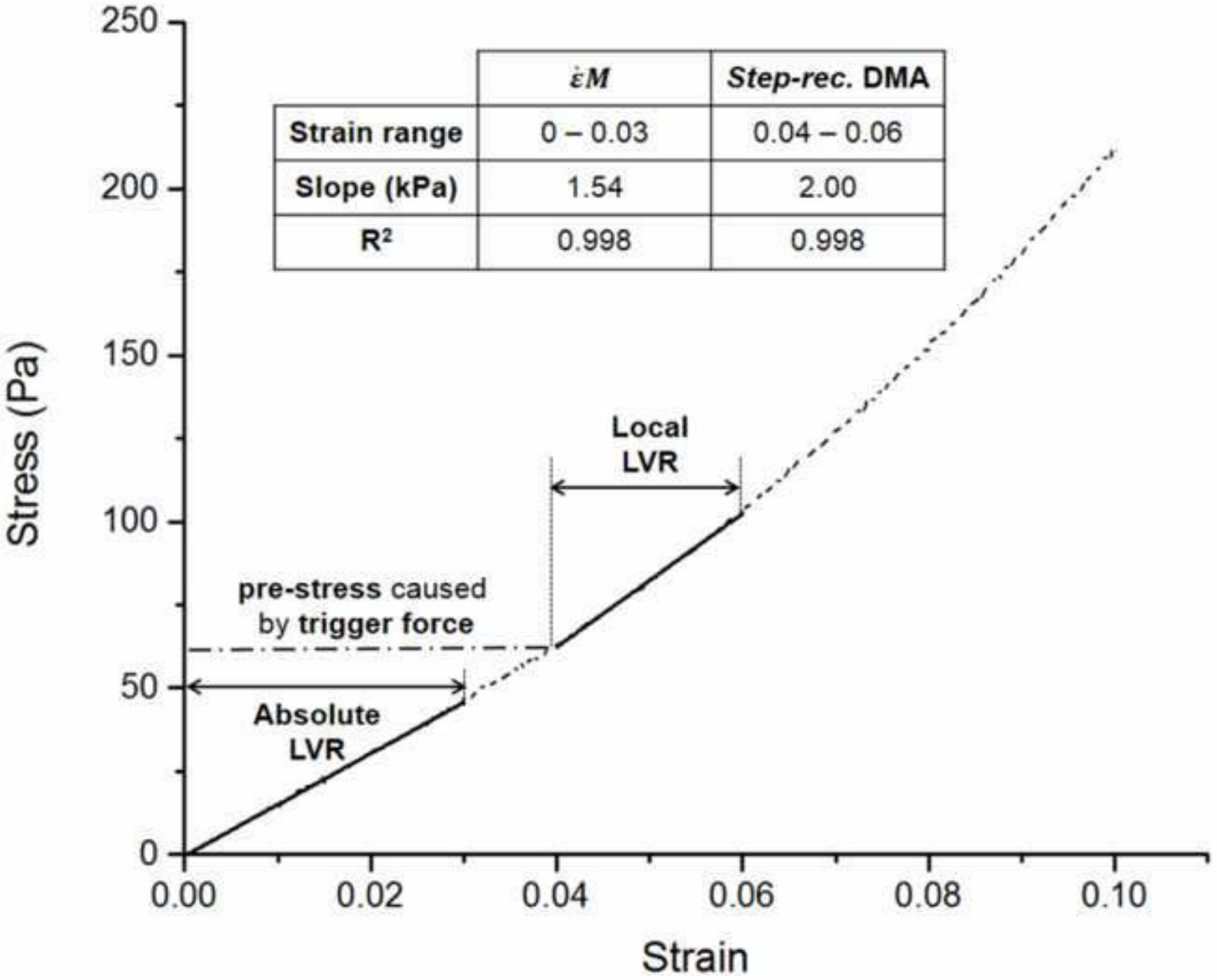


Figure 4

Figure 5



	$\dot{\varepsilon}M$	*Step-rec.* DMA
Strain range	0 – 0.03	0.04 – 0.06
Slope (kPa)	1.54	2.00
R^2	0.998	0.998