Journal of the Mechanical Behavior of Biomedical Materials 84 (2018) 198–207

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Energy dissipation in quasi-linear viscoelastic tissues, cells, and extracellular matrix

Behzad Babaei[a], A.J. Velasquez-Mao[b], Kenneth M. Pryse[c], William B. McConnaughey[c], Elliot L. Elson[c], Guy M. Genin[d,*]

[a] Neuroscience Research Australia, Randwick, Australia
[b] UC Berkeley and UC San Francisco Graduate Program in Bioengineering, San Francisco, CA, USA
[c] Department of Biochemistry & Molecular Biophysics, Washington University School of Medicine, St. Louis, MO, USA
[d] NSF Science and Technology Center for Engineering Mechanobiology, Washington University in St. Louis, St. Louis, MO, USA

ARTICLE INFO

Keywords:
Energy dissipation
Strain rate dependence
Viscoelasticity
Fung quasi-linear viscoelastic model
Engineered tissue constructs

ABSTRACT

Characterizing how a tissue's constituents give rise to its viscoelasticity is important for uncovering how hidden timescales underlie multiscale biomechanics. These constituents are viscoelastic in nature, and their mechanics must typically be assessed from the uniaxial behavior of a tissue. Confounding the challenge is that tissue viscoelasticity is typically associated with nonlinear elastic responses. Here, we experimentally assessed how fibroblasts and extracellular matrix (ECM) within engineered tissue constructs give rise to the nonlinear viscoelastic responses of a tissue. We applied a constant strain rate, "triangular-wave" loading and interpreted responses using the Fung quasi-linear viscoelastic (QLV) material model. Although the Fung QLV model has several well-known weaknesses, it was well suited to the behaviors of the tissue constructs, cells, and ECM tested. Cells showed relatively high damping over certain loading frequency ranges. Analysis revealed that, even in cases where the Fung QLV model provided an excellent fit to data, the the time constant derived from the model was not in general a material parameter. Results have implications for design of protocols for the mechanical characterization of biological materials, and for the mechanobiology of cells within viscoelastic tissues.

1. Introduction

Connective tissue structures throughout the body are constantly under dynamic loading (Butler et al., 2000; Ditsios et al., 2002; Kim et al., 2008). The responses of these tissues to dynamic loads are therefore central to their structural function, and data are needed to fit and validate the many multiscale models of how these responses arise from those of a tissue's constituents (Genin et al., 2017). The broad literature of techniques for characterizing viscoelastic behavior of tissues focuses on uniaxial tests such as creep or relaxation testing, due in part to the aligned, fibrous structure common amongst tissues (Purslow et al., 1998; Jamison et al., 1968; Hudnut et al., 2017; Castile et al., 2016). Because nonlinearity can arise from both time-independent and time-dependent sources, isolating strain-rate dependence of viscoelasticity is challenging (Sakamoto et al., 2017; Lokshin and Lanir, 2009; Li et al., 2017).

Here, we developed and applied the quasi-linear viscoelastic (QLV) framework for a material subjected to a triangular-wave strain loading pulse (Fig. 1) to study the damping of a collagenous tissue construct. The Fung QLV material model is known to be highly limited. Although available data indicate that the Fung QLV model's separation of nonlinear elastic effects from linear, strain independent relaxation effects is often acceptable over a certain range of conditions (Babaei et al., 2015a), no known material follows Fung QLV behavior faithfully across all loadings (Lakes and Vanderby, 1999; Provenzano et al., 2001, 2002; Ciarletta et al., 2006; Duenwald et al., 2010). Many fixes and extensions of the Fung QLV model exist (Provenzano et al., 2001; Pryse et al., 2003; Nekouzadeh et al., 2007). However, the Fung QLV model is nearly a standard starting point for the analysis of nonlinear biological materials (Zou and Zhang, 2011; Thomopoulos and Genin, 2012; Giles et al., 2007; Bischoff, 2006; Doehring et al., 2004; Kwan et al., 1993; Carew et al., 1999; Sverdlik and Lanir, 2002). We therefore focused the treatment of the subject on the Fung QLV model itself.

Although a very general framework exists for modeling nonlinear viscoelastic behavior (Coleman and Noll, 1961), a general framework for specific biological tissues remains elusive. Fung's QLV model is attractive as a starting point because it identifies a class of quasi-linearity that is appropriate for many biological tissues (Nekouzadeh et al.,

* Corresponding author.
E-mail address: genin@wustl.edu (G.M. Genin).

https://doi.org/10.1016/j.jmbbm.2018.05.011
Received 23 February 2018; Received in revised form 1 May 2018; Accepted 7 May 2018

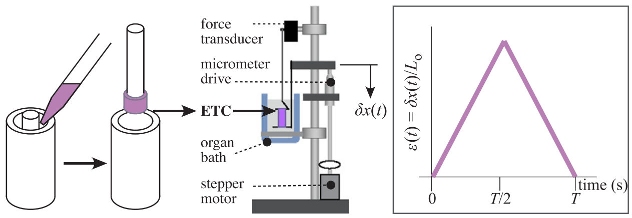

Fig. 1. Annular engineered tissue constructs (ETCs) were stretched in a custom stretching apparatus using a triangular-wave (sawtooth) strain profile. Portions of this were figure reproduced with permission from Elson and Genin (2016).

2007). When applying it, one must bear in mind the restrictions it imposes due to the "box-shaped" temporal relaxation function that Fung proposed in his text (Fung, 2013). The width of this spectrum typically cannot be fit with confidence, which complicates the comparison of materials (Babaei et al., 2015a).

To determine whether the Fung QLV is a reasonable model, a spectral approach can be used such as the discrete Fung QLV (DQLV) approach of Babaei et al. (2015a). This identifies ranges of discrete time constants over which the Fung QLV model is a reasonable approximation to the material response. Identifying when the box-shaped relaxation function is inadequate poses difficulty (Thomopoulos et al., 2003; Sauren and Rousseau, 1983) because, with this box spectrum, the Fung QLV model can fit relaxation data for materials whose responses to dynamic loading it would fail to predict (Iatridis et al., 1997; Anderson et al., 1991). However, in the current study, we observed that for each triangle loading cycle, only one time constant and one corresponding damping coefficient was expressed strongly in the specimens, reducing the modeling complexity required for interpreting the data. The current testing protocol enabled samples to relax adequately between loading cycles. Both the Fung QLV and DQLV models were able to fit the data properly, and we therefore focussed on the former, and on a range of loading rates that are relevant to slower physiological processes.

Theories describing instantaneous stress response and energy dissipation of a standard linear viscoelastic solid subject to such loadings are well established, although the definitive works of Tschoegl (1981) and Yang and Chen (1982) are surprisingly recent. The application of this and other methods of estimating hysteresis in biological structures is widespread (Nyman et al., 2007; Koolstra et al., 2007; LaCroix et al., 2013). However, even in the linear sense, the sensitivity of hysteretic energy dissipation for biological structures across the spectrum of loading periods has not been explored in detail. Given the time constants of connective tissues, knowledge of dissipated energy as a function of loading time can provide a better understanding of, for example, the loading conditions at which injury resistance might be optimal, or those at which cells might be more sensitive to mechanical stimuli. We and others have observed that hysteresis observed in loading/unloading curves measured on engineered tissue constructs is not well represented in terms of linear viscoelasticity (Nekouzadeh et al., 2007).

From a clinical perspective, models of connective tissue-based support structures are needed to understand physiological degeneration, especially at the slow loading rates associated with viscoelastic relaxation responses of tissues (Karamanidis and Arampatzis, 2005; Hajdu et al., 1990; Rotsch and Radmacher, 2000). In tissue engineering, such models are needed to engineer tissues that mimic structures within the body (Vunjak-Novakovic et al., 1999). Mechanical standards to evaluate the performance of artificially grown structures prior to and after implantation represent a longstanding need (Butler et al., 2000), especially given the range of work focused on altering the viscoelastic properties of engineered tissue structures by manipulating scaffolding material, varying matrix protein compositions, and external static and dynamic stresses on tissues during formation (Feng et al., 2006; Courtney et al., 2006; von der Mark et al., 2010; Thomopoulos et al., 2011; Huang et al., 2017; Yeh et al., 2017; Li and Zhang, 2014). However, assessing and predicting how these structures will perform requires further study of the dynamic responses to different loading patterns and how this behavior changes as a function of the loading period.

From the standpoint of cellular mechanobiology, key questions are the degree to which cells and extracellular matrix (ECM) contribute to the overall mechanical function of a tissue, including its hysteretic behavior, and how mechanical signals travel through ECM to reach cells (Shakiba et al., 2017). Estimates of the viscoelastic responses of cells have been made in using artificial tissue constructs, but these have been limited to linear viscoelasticity (Babaei et al., 2016). In this work, we extended these results to distinguish the Fung QLV behaviors of cells and ECMs in such constructs.

The work focused on triangular-wave stretching of tissue constructs, rather than frequently-used sinusoidal stretching, for two reasons. First, whereas for a sinusoidal excitation, the nominal strain rate alters sinusoidally over each loading cycle, the magnitude of the nominal strain rate is constant for a triangular-wave excitation. Thus, to study the effect of the strain rate on the dynamic responses of a biological material, a triangular-wave excitation is a better choice. Second, because biological materials typically behave non-linearly (Storm et al., 2005; Dhume and Barocas, 2017), these varying strain rates must be convolved into model fitting for something other than infinitesimal straining. Although these nonlinear responses can be estimated by a test protocol with a series of infinitesimal relaxation increments (Pryse et al., 2003), we show that the triangular waveform has certain advantages. Therefore, we offered a mathematical approach for modeling the viscoelastic response to triangular-wave excitation, which allows practical experimentation under a constant strain rate. We then applied this model to simulate and interpret the behavior of engineered tissue constructs (ETCs), including the dependence of energy dissipation on loading rate in ETCs, remodeled collagen ECMs, and fibroblasts.

2. Theory

We studied the response of a Fung QLV material to a single triangular-wave stretching (Fig. 1). An assumption was that the material constants do not change substantially from one loading cycle to the next. Although this is never fully the case for a biological tissue, it can be achieved to a reasonable approximation for the tissues of interest through application of the appropriate preconditioning protocols (Wagenseil et al., 2003; Cohen et al., 2008; Marquez et al., 2006b). In this section, we begin by reviewing linear elastic, linear viscoelastic, and quasi-linear material responses, then apply these to develop expressions for the damping of these materials when subjected to a triangular-wave loading pulse.

2.1. Background: hereditary viscoelastic materials

The response of a hereditary or non-aging viscoelastic material to a one-dimensional straining can be represented by the Stieltjes convolution integral (Stieltjes, 1894; Lockett, 1972):

$$\sigma(t) = \int_0^t \phi(t-u)\frac{\partial \varepsilon}{\partial u}du \tag{2.1}$$

where $\sigma(t)$ is engineering stress, t is time, ε is linearized strain, u is a dummy parameter, and $\phi(t)$ is a material modulus function. $\phi(t)$ can represent a linear elastic solid with the choice $\phi = E_0$, in which E_0 is the solid's elastic modulus; it can represent a Newtonian fluid with the choice $\phi(t) = \eta\delta(t)$, where $\delta(t)$ is Dirac's delta function and η is the fluid's viscosity; and it can represent a viscoelastic material possessing both elastic and viscous properties with $\phi(t)$ chosen as a generalized function that captures the whole spectrum of material behaviors. A common form for representing this is the generalized Maxwell (Maxwell-Weichert) linear viscoelastic material, comprised of a linear spring