ARTICLE IN PRESS

Journal of Biomechanics ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Short communication

# Development of a bilayer ring system for achieving high strain in commercial rheometers

Michael B. Christensen [a,b,*], Jeffrey C. Wolchok [c], Sarah A. Klemuk [a,d], Ingo R. Titze [a]

[a] The National Center for Voice and Speech, University of Utah, 136 South Main Street, Suite 320, Salt Lake City, UT 84101, USA
[b] Keck Center for Tissue Engineering, Department of Bioengineering, University of Utah, 36 South Wasatch Drive, Rm 3100, Salt Lake City, UT 84112, USA
[c] Department of Biomedical Engineering, University of Arkansas, 317 Engineering Hall, Fayetteville, AR 72701, USA
[d] Department of Communication Sciences and Disorders, University of Iowa, 324 SHC, Iowa City, IA 52242, USA

ARTICLE INFO

*Article history:*
Accepted 13 July 2015

*Keywords:*
Elastic modulus
Viscous modulus
Rheometry
Resonance frequency

ABSTRACT

Mechanical stimulation of cell cultures has been shown be an effective means of enhancing ECM production. ECM produced from vocal fold fibroblast cultures has the potential for therapeutic use for vocal fold repair. However, current bioreactor designs generally fail to produce physiological relevant frequency and strain values. Here we present an approach for using commercial oscillatory rheometers and an elastic ring bilayer system to produce physiologically relevant strain values at frequencies in the range of 20–100 Hz. We demonstrate the ability to target specific strain and frequency values by manipulating system parameters, and also show that it is possible to maintain high oscillatory strains for extended periods of time. Such a system could be used to mechanically stimulate cell cultures contained within gel carrier systems and has the potential to be extended to other applications requiring high strains at low frequencies.



## 1. Introduction

Cell-derived extracellular matrix (ECM) has therapeutic potential for the treatment of vocal fold injury. Collection of ECM may be accomplished by culturing cells *in vitro*. Ideally, ECM would exhibit properties similar to native vocal fold tissue. Physiologically relevant mechanical stimulation of cell cultures may provide a way to influence ECM properties to more closely match those of native ECM, and has been shown to be an effective way of enhancing ECM production (Chiquet et al., 2003). However, previous research using bioreactors to mimic the mechanical properties of the vocal fold have used synthetic scaffolding as a cell carrier (Gaston et al., 2012; Titze et al., 2004a; Wolchok et al., 2009). Such materials may lack the ability to safely degrade in the body and may, even if they are resorbable, cause an inflammatory reaction when implanted. Therefore, methods of mechanically stimulating cultures using more natural materials, such as hyaluronic acid (HA) gels, as cell carriers may offer greater therapeutic potential. Modifications made to standard commercial rheometer setups may allow for the stimulation of such cultures.

While there are several methods which may be employed to measure the biomechanical properties of the vocal fold (Miri, 2014), rheometry is the common method used to measure frequency dependent viscoelastic properties. Oscillatory tissue deformation in a torsional rheometer is facilitated by the elastic component of the tissue by providing a restoring force to assist the motor in periodic acceleration and deceleration. A mechanical resonance between motor-shaft inertia and material elasticity can also be exploited (Titze et al., 2004b), in which large strains can be achieved in rheometers designed for only small strain applications.

The elastic properties of most materials are sufficient for achieving high strains, even when the amount of torque available is small (Klemuk et al., 2008). However, when investigating more fluid or gel-like materials for vocal fold applications, previous results using commercial rheometers have shown a limited ability to achieve large strains (above ~20%) or high frequencies (above 20 Hz) (Chan et al., 2001; Chan and Titze, 1998; Kutty and Webb, 2009). This limitation currently presents an obstacle for stimulation of cells under physiological conditions using rheometers.

Here we describe an adaptation to exploit resonance between a secondary material and the inertia of the motor-shaft assembly such that large strains and high frequencies can be achieved with primary materials whose lack of a restoring force would normally prevent such operations. The application of this adaptation may provide a means to use standard rheometer setups in a bioreactor role to mechanically stimulate cell cultures.

* Corresponding author at: 36 South Wasatch Drive, Rm 3100, SMBB, Salt Lake City, UT 84112, USA. Tel.: +1 801 587 0598; fax: +1 801 581 8692.
*E-mail address:* ben.christensen@utah.edu (M.B. Christensen).

http://dx.doi.org/10.1016/j.jbiomech.2015.07.011
0021-9290/

Please cite this article as: Christensen, M.B., et al., Development of a bilayer ring system for achieving high strain in commercial rheometers. Journal of Biomechanics (2015), http://dx.doi.org/10.1016/j.jbiomech.2015.07.011

## 2. Calculation and control of resonance frequency

Given that rheometer motor-shaft assemblies are usually designed to have negligible stiffness between plates under no-load conditions, a mechanical resonance frequency occurs only when the test material between the plates has stiffness. Since this material stiffness is generally unknown (it is the object of measurement), the resonance frequency is not under direct control. However, by adding an elastic material of known properties to create a concentric bilayer system, a predictable resonance frequency can be created.

Fig. 1 shows a concentric bilayer system consisting of an inner material with shear properties $G_1$' and $G_1$'' surrounded by an elastic ring with shear properties $G_2$' and $G_2$''. If both materials adhere to the plates, the torque ($T$) developed by the material at the top plate boundary is

$$T = \int_0^{r_1} \tau_{\theta z} dA \cdot r + \int_{r_1}^{r_2} \tau_{\theta z} dA \cdot r, \tag{1}$$

where $\tau_{\theta z}$ is the shear stress on the top surface, $dA$ is a differential ring area, $r$ is the radius to the differential ring area, and $r_1$ and $r_2$ are the radii of the inner and outer materials respectively. For linear viscoelasticity under sinusoidal displacement $\theta$ and a gap $d$ between the plates, the complex stress–strain relation is (Macosko, 1994)

$$\bar{\tau}_{\theta z} = G'\frac{r\theta}{d} + iG''\frac{r\theta}{d}, \tag{2}$$

and the differential area is

$$dA = 2\pi r dr \tag{3}$$

substituting these quantities into Eq. (1) yields the complex torque

$$\bar{T} = \frac{2\pi}{d}(G'_1 + iG''_1)\theta \int_0^{r_1} r^3 dr + \frac{2\pi}{d}(G'_2 + iG''_2)\theta \int_{r_1}^{r_2} r^3 dr \tag{4}$$

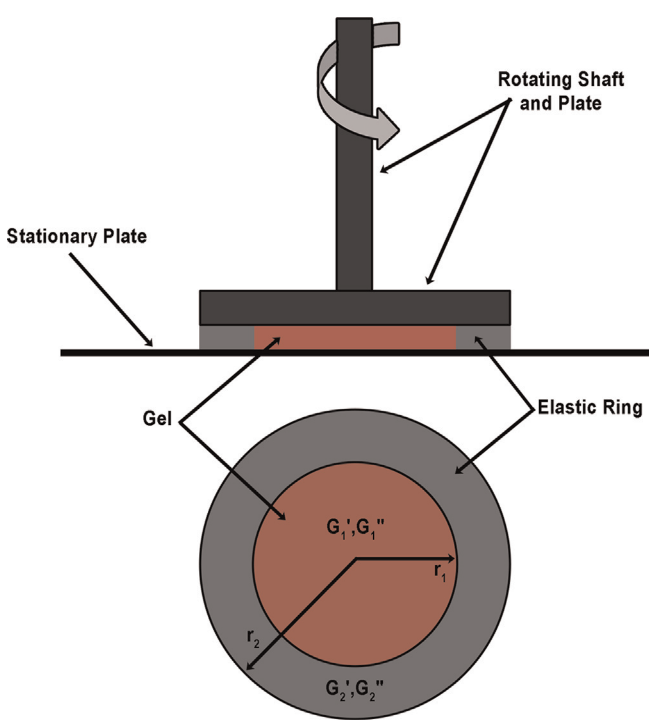


**Fig. 1.** Schematic of concentric bilayer system. Subscipt 1 denotes the inner material of unknown properties, while subscript 2 denotes the elastic material of known properties.

after evaluating the integrals,

$$\bar{T} = \frac{\pi\theta}{2d}\left[r_1^4 G'_1 + \left(r_2^4 - r_1^4\right)G'_2\right] + i\frac{\pi\theta}{2d}\left[r_1^4 G''_1 + \left(r_2^4 - r_1^4\right)G''_2\right] \tag{5}$$

$$\bar{T} = \frac{\pi\theta}{2d}(G' + iG'')r_2^4, \tag{6}$$

where equivalent single-layer elastic and viscous shear moduli are defined as

$$G' = \left[r_1^4 G'_1 + \left(r_2^4 - r_1^4\right)G'_2\right]/r_2^4 \tag{7}$$

$$G'' = \left[r_1^4 G''_1 + \left(r_2^4 - r_1^4\right)G''_2\right]/r_2^4. \tag{8}$$

Note that if $r_1=0$, the outer ring becomes a full disk with $G'=G_2'$ and $G''=G_2''$. At the other extreme, if $r_1=r_2$, $G'=G_1'$ and $G''=G_1''$.

The equation of motion for the rheometer system includes the inertia $I$,

$$\bar{T}_0 = \bar{T} - I\omega^2\theta = \frac{\pi\theta}{2d}(G' + iG'')r_2^4 - I\omega^2\theta, \tag{9}$$

where $\bar{T}_0$ is the torque applied by the motor, $I$ is the moment of inertia of the motor-shaft-plate combination, and $\omega$ is the angular frequency of the plate. The frequency response of the system is taken to be the ratio of the angular displacement, $\theta$, to the applied torque,

$$\theta/\bar{T}_0 = \frac{1}{\pi G' r_2^4/2d - I\omega^2 + i\pi G'' r_2^4/2d}. \tag{10}$$

A resonance frequency occurs when the first two terms in the denominator cancel each other, which leads to

$$f_0 = \frac{r_2^2}{2\pi}\sqrt{\frac{\pi G'}{2dI}}. \tag{11}$$

All variables in this equation are known except $G'$. However, if $\left(r_2^4 - r_1^4\right)G'_2 \gg r_1^4 G'_1$ in Eq. (7), then all variables are known and the resonance frequency becomes

$$f_0 \approx \frac{1}{2\pi}\sqrt{\frac{\pi\left(r_2^4 - r_1^4\right)G'_2}{2dI}} \tag{12}$$

## 3. Theoretical response curves

For conditioning of cell systems for vocal fold repair, shear strains in the range of 0.1–0.5 are desirable (Titze et al., 2003). Given that strain varies radially in the material, a convention has been established to choose the 75% value of the sample radius as a representative strain for the test material (Macosko, 1994). Thus, if

$$\gamma = \frac{0.75 r_1}{d} \tag{13}$$

the strain response from Eq. (10) can be written as

$$\gamma/T_0 = \frac{1.5 r_1/G'\pi r_2^4}{1 - (\omega/\omega_0)^2 + i(G''/G')} \tag{14}$$

where $\omega_0 = 2\pi f_0$.

Fig. 2 shows theoretical response curves for strain $\gamma$ when $d$, $r_1$, $G_2'$, and $G_2''/G_2'$ are varied. The nominal parameter values selected were $I$=3.5E-05 kg-m$^2$, $r_1$=15 mm, $r_2$=30 mm, $d$=1.5 mm, $G_1'$=1 kPa, $G_1''$=1 kPa, $G_2'$=30 kPa, and $G_2''/G_2'$=0.15. With these