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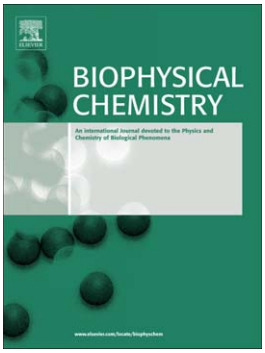

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PII: S0301-4622(11)00077-9
DOI: doi: 10.1016/j.bpc.2011.04.008
Reference: BIOCHE 5504

To appear in: *Biophysical Chemistry*

Received date: 5 March 2011
Revised date: 7 April 2011
Accepted date: 7 April 2011

Please cite this article as: Laurence A. Belfiore, Michael L. Floren, Alexandre T. Paulino, Carol J. Belfiore, Stress-Sensitive Tissue Regeneration in Viscoelastic Biomaterials Subjected to Modulated Tensile Strain, *Biophysical Chemistry* (2011), doi: 10.1016/j.bpc.2011.04.008

Stress-Sensitive Tissue Regeneration in Viscoelastic Biomaterials Subjected to Modulated Tensile Strain

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Abstract

This research contribution addresses the mechanochemistry of intra-tissue mass transfer for nutrients, oxygen, growth factors, and other essential ingredients that anchorage-dependent cells require for successful proliferation on biocompatible surfaces. The unsteady state reaction-diffusion equation (i.e., modified diffusion equation) is solved according to the von Kármán-Pohlhausen integral method of boundary layer analysis when nutrient consumption and tissue regeneration are stimulated by harmonically imposed stress. The mass balance with diffusion and stress-sensitive kinetics represents a rare example where the Damköhler and Deborah numbers appear together in an effort to simulate the development of mass transfer boundary layers in porous viscoelastic biomaterials. The Boltzmann superposition integral is employed to calculate time-dependent strain in terms of the real and imaginary components of dynamic compliance for viscoelastic solids that transmit harmonic excitation to anchorage-dependent cells. Rates of nutrient consumption under stress-free conditions are described by third-order kinetics which include local mass densities of nutrients, oxygen, and attached cells that maintain dynamic equilibrium with active protein sites in the porous matrix. Thinner nutrient mass transfer boundary layers are stabilized at shorter dimensionless diffusion times when the stress-free intra-tissue Damköhler number increases above its initial-condition-sensitive critical value. The critical stress-sensitive intra-tissue Damköhler number, above which it is necessary to consider the effect of harmonic strain on nutrient consumption and tissue regeneration, is proportional to the Deborah number and corresponds to a larger fraction of the stress-free intra-tissue Damköhler number in rigid biomaterials.

Keywords: Fick's 2nd law, unsteady state diffusion, modified diffusion equation, regenerative tissue, stress-sensitive kinetics, Boltzmann superposition integral, intra-tissue Damköhler number, stress-sensitive Damköhler number, Deborah number, von Kármán-Pohlhausen profile method, mass transfer boundary layer thickness.

Submitted to *Biophysical Chemistry;* March 5th, 2011: revision submitted on April 7th, 2011.
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This manuscript commemorates the 100th anniversary of the birth of Olivia DeVito Belfiore on June 18th, 1911, and the death of her mother upon childbirth. It is submitted in memory of my best childhood friend, Frank Daniel DeVito, whose 56th birthday would have occurred on Dec. 6th, 2010.

Introduction

The material properties of biological tissue arise from nanoscale and microscale architecture of sub-cellular, cellular, and extracellular networks [1]. Living cells grow and exert their activities while embedded in a dense, complex extracellular matrix. This matrix contains an array of structural and directional cues that guide and support the morphogenesis of multi-cellular structures such as tissues and organs. Dynamic models with the potential to predict macroscale behavior from the microscale continuum are useful to describe underlying multi-scale processes that occur when tissues are stimulated by mechanical stress. Fractional (non-integer order) calculus has been applied to develop models that consider these biological events [1]. The mechano-biology of tendons requires a complete understanding of its liquid constituents in the presence of stress, because tendon viscoelasticity depends on (i) water content and (ii) physico-chemical influence on anisotropic intra-tendon diffusion [2]. In some cases, wave propagation described by the reaction-diffusion equation initiates deformation in cardiac tissue via a process known as mechano-electrical feedback [3]. Exact and approximate solutions of the one-dimensional reaction-diffusion equation describe oxygen delivery by the microcirculation system and oxygen diffusion/consumption in muscle tissue when oscillatory boundary conditions mimic local blood flow regulation as a function of oxygen concentration [4].

Viscous shear at fluid-solid interfaces [5,6] and centrifugal-force-induced compressive stress [7] have been employed previously to stimulate endothelial cell and bone cell proliferation, respectively, in chemisorbed monolayers on protein-coated surfaces. When nutrient media flow past active surfaces that contain anchorage-dependent cells, simple 1-dimensional Newtonian fluid velocity profiles and the magnitude of the velocity gradient tensor are required to construct stress-kinetic reciprocal couplings that obey Curie's theorem in nonequilibrium thermodynamics [5,6,8,9]. In this study, strain-activated tissue regeneration is stimulated by subjecting viscoelastic biomaterials that contain uniformly dispersed anchorage-dependent cells to harmonic tensile stress. The formalism for scalar cross-phenomena originates from a consideration of the mechanochemistry of materials [10] and the corresponding rate of entropy generation in solids [11], but slight modification is necessary to include a contribution from time-independent strain to stress-sensitive reactions. Hence, scalar stress-kinetic couplings are reformulated in terms of the magnitude of the 2nd-rank strain tensor, not the velocity gradient tensor or the corresponding symmetric rate-of-strain tensor that is typical for fluids. The strain-energy function represents another option to characterize the effect of deformation on biochemical kinetics [11,12]. A *strain-energy-dependent* source term for bone cell proliferation that monitors tissue rigidity was proposed by Harrigan and Hamilton [13] which becomes activated when strain energy density increases above a predetermined threshold [12]. This threshold is analogous to the identification of a critical

value of the stress-sensitive intra-tissue Damköhler number, defined herein, above which it is necessary to consider the effect of harmonic stress on nutrient consumption and tissue regeneration. The Boltzmann superposition integral for time-dependent strain [14] contains elastic and viscous contributions to deformation in porous biomaterials, and harmonic stress excitation introduces storage and loss compliances in the effect of strain on proposed kinetic models. Optimal synthetic scaffolds that exhibit ideal combinations of physical, chemical, and biological stimuli pose a bioengineering design dilemma.

Motivation and Strategy

This research contribution analyzes predictions from the *reaction-diffusion equation* in biological systems that respond to deformation. The overall objective is to develop guidelines that quantify the importance strain-catalyzed rates of nutrient consumption when anchorage-dependent cells are stimulated in viscoelastic biomaterials. Nutrients diffuse inward from the external biomaterial interface to support cell proliferation, and the mass transfer boundary layer thickness in the presence and absence of stress is used as a metric to evaluate tissue regeneration. Hence, nutrient boundary layers decrease in thickness when harmonic excitation is transmitted to attached cells in a porous matrix. The next section provides a phenomenological explanation for enhanced rates of nutrient consumption by anchorage-dependent cells via *symmetry-breaking* phenomena [15] as a consequence of stress imbalance. The *reaction-diffusion equation* is satisfied throughout the nutrient boundary layer with assistance from the von Kármán-Pohlhausen integral method of analysis that yields a time-dependent expression for boundary layer thickness, δ_{MTBLT}, which is affected by stress-free and stress-sensitive rates of consumption. Numerical results in Figures 2-4 might be useful to design compliant biomaterials for tissue regeneration such that nutrients, oxygen, and growth factors exist throughout the matrix under quasi-steady-state conditions to support cell proliferation and sustainability. Mathematical solution of the *reaction-diffusion equation* is performed using dimensionless variables and parameters, with time t and spatial coordinate x transformed according to the *combination-of-variables* method of analysis. The *intra-tissue Damköhler number* emerges as the most important parameter governing the thickness of the nutrient boundary layer. Since nutrients are consumed according to stress-free and stress-sensitive kinetic pathways, an *intra-tissue Damköhler number* is defined for each mechanism. The strain-catalyzed mechanism of nutrient consumption is formulated in terms of the real and imaginary components of dynamic compliance for viscoelastic solids, as described in the next section. Consequently, the *Deborah number* appears as a dimensionless parameter in the *reaction-diffusion equation,* and in the numerical results for δ_{MTBLT}. The *Deborah* and *Damköhler numbers* have not appeared together in previous examples of the *reaction-diffusion equation* because strain-catalyzed (or stress-sensitive) nutrient consumption has not received much attention and the corresponding dimensionless equations have not been analyzed. These are important modeling issues in

the field of *mechanobiology,* allowing cells in a regenerative matrix to proliferate at their maximum potential via mechanical stimulation. The magnitude and frequency of mechanical excitation are the focus of current biomaterials research. This investigation combines elements of (i) transport phenomena, (ii) strain-catalyzed reaction kinetics, and (iii) viscoelastic biomaterials to identify critical values of the stress-sensitive *intra-tissue Damköhler number,* above which tissue engineering design should consider the effect of biomaterial deformation on cell proliferation and tissue regeneration.

Magnitude of the strain tensor for viscoelastic solids subjected to harmonic stress via the Boltzmann superposition integral

The cell/nutrient-medium interface is analogous to a gel-liquid boundary, and cell growth and deformation produce elastic stresses that depend on the mechanical properties of the cell, according to the laws of continuum mechanics [15]. Spherical cells that grow axisymmetrically generate a normal tensile stress imbalance on their outer surface, in the tangential direction (i.e., $\sigma_{\Theta\Theta}$), when symmetry is perturbed (i.e., *symmetry breaking*) as a consequence of natural fluctuations and cell motility. The magnitude of these elastic stresses is proportional to the thickness of the cell's outer "comet-shaped" surface that develops [15]. The state of deformation and the macroscopic stress distribution "catalyze" rates of nutrient consumption by anchorage-dependent cells in the regenerative process. Kinetic models are proposed herein [i.e., see Eq. (4)] that contain an additional contribution due to viscoelastic deformation of the biomaterial support. The scalar rate of nutrient consumption is coupled to the *magnitude of the second-rank strain tensor* [12,13], defined by the square-root of the double-dot product of the strain tensor with its transpose [16]. Harmonic tensile stress is given by;

$$\sigma(t;\omega) = \sigma_{DC} + \sigma_{AC}\cos(\omega t) \qquad (1)$$

such that $\sigma_{AC} < \sigma_{DC}$, and the time-dependent creep compliance for an infinite spectrum of Voigt elements in series [14] is expressed in terms of the relaxation time distribution function, $J_D(\lambda)$;

$$J_C(t-\Theta) = \sum_{i=1}^{N} \frac{1}{E_i}\left\{1-\exp\left[-\frac{(t-\Theta)}{\lambda_i(T)}\right]\right\} \underset{N\to\infty}{\Rightarrow} \int_{\lambda=0}^{\infty} J_D(\lambda)\left\{1-\exp\left[-\frac{(t-\Theta)}{\lambda}\right]\right\} d\lambda \qquad (2)$$

The Boltzmann superposition integral for time-dependent strain [14] is employed to evaluate the magnitude of the strain tensor in terms of the storage $J'(\omega)$ and loss $J''(\omega)$ components of dynamic creep compliance at excitation frequency ω.

$$\text{Magnitude of the strain tensor; } |\gamma(t;\omega)| = \sqrt{\frac{1}{2}\{\gamma\}:\{\gamma\}^T}$$

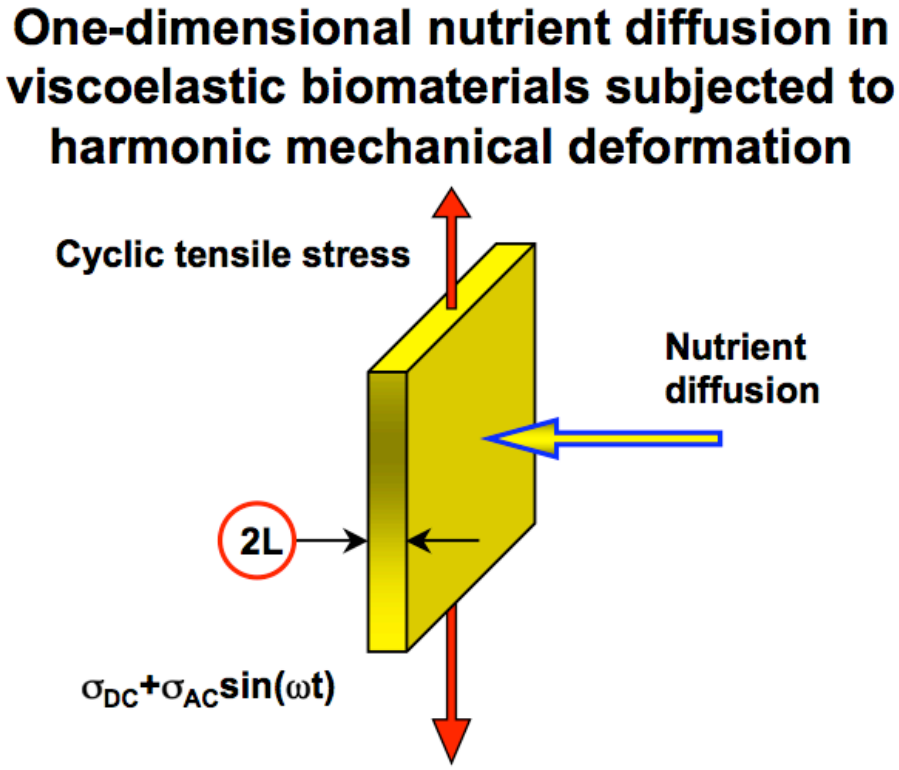
$$= \frac{1}{\sqrt{2}}\sqrt{\left[\int_{\Theta\Rightarrow-\infty}^{t} J_C(t-\Theta)\left\{\frac{\partial\sigma(\Theta;\omega)}{\partial\Theta}\right\}_{\omega} d\Theta\right]^2}$$

$$= \frac{\sigma_{AC}}{\sqrt{2}}\sqrt{\{J'(\omega)\cos(\omega t) + J''(\omega)\sin(\omega t)\}^2} \qquad (3)$$

$$J'(\omega) = \int_{x=0}^{\infty} J_D(x)\frac{1}{1+(\omega x)^2}dx \overset{J_D(x)=\frac{1}{E}\delta(x-\lambda)}{\Rightarrow} \frac{1}{E}\left\{\frac{1}{1+De^2}\right\}$$

$$J''(\omega) = \int_{x=0}^{\infty} J_D(x)\frac{\omega x}{1+(\omega x)^2}dx \overset{J_D(x)=\frac{1}{E}\delta(x-\lambda)}{\Rightarrow} \frac{1}{E}\left\{\frac{De}{1+De^2}\right\}$$

As illustrated in Figure#1, the coordinate direction in which deformation occurs is transverse to the diffusional flux of nutrients and other essential ingredients required for cell proliferation and tissue regeneration.



Figure#1

Schematic representation of porous biomaterials subjected to harmonic mechanical stimulation, with one-dimensional nutrient diffusion inward along the thinnest tissue dimension to support cell proliferation and sustainability.

It is not unreasonable to (i) identify ω as the dominant frequency in the power spectrum when one considers the coupling between cardiac and respiratory oscillators superimposed on random noise [17,18], and (ii) design experiments with significant overlap between these oscillations and the excitation frequency that stimulates tissue regeneration. The scaling of time in viscoelasticity (i.e., material response time λ relative to a characteristic time for the deformation process) is accomplished via the Deborah number De, which is given by $\omega\lambda(T)$ for a one-time-constant model with static compliance 1/E, where E is the static modulus of the elastic element in the viscoelastic model [14]. The factor of 0.5 under the square-root sign in Eq. (3) guarantees that the magnitude of the symmetric 2nd-rank strain tensor reduces to its only independent off-diagonal element when all other elements vanish [16].

Stress-free and stress-sensitive rates of nutrient consumption in viscoelastic biomaterials

It is necessary to construct mathematically correct stress-kinetic reciprocal relations that describe scalar cross-phenomena [5] when the state of deformation in viscoelastic biomaterials is coupled to the rate of nutrient consumption and cell proliferation in regenerative tissue. This phenomenon could be significant at small length scales in cellular "micro-reactors" (i.e., on the order of isolated cells with a diameter of 10 μm that are attached to a regenerative matrix). The magnitude of the strain tensor in Eq. (3) is employed to accomplish this coupling, according to Curie's theorem in nonequilibrium thermodynamics [5,8,9] when the rate of entropy generation contains fluxes and force whose tensorial ranks differ by an even integer (i.e., in this case, two). This formalism is employed to modify scalar rates of reaction when the medium is subjected to tensile or compressive deformation. For mechanochemical systems that are not too far removed from *equilibrium,* homogeneous rates of nutrient consumption are written in the following form;

$$R_{\substack{\text{homogeneous}\\ \text{nutrient}\\ \text{consumption}}} = k_{stress-free}\rho_{nutrient}\rho_{oxygen}\rho_{cells} + \kappa_{stress}\frac{\sigma_{AC}}{\sqrt{2}}\sqrt{\left\{J'(\omega)\cos(\omega t) + J''(\omega)\sin(\omega t)\right\}^2} \qquad (4)$$

where the scalar Onsager coupling coefficient κ_{stress} has dimensions of nutrient mass per volume of the viscoelastic biomaterial per time. The form of the *stress history function* in the Boltzmann superposition integral for time-dependent strain [i.e., see Eq. (3)] eliminates effects from time-independent stress σ_{DC} on the rate of nutrient consumption. However, the fact that harmonic stress always induces tensile strain (i.e., since $\sigma_{DC} > \sigma_{AC}$) has an implicit effect on scalar stress-kinetic coupling. The second term on the right side of Eq. (4) has been modified phenomenologically via the magnitude of the strain tensor, instead of employing the velocity gradient tensor or the symmetric rate-of-strain tensor, as

suggested by the linear laws of irreversible thermodynamics for viscous fluids that focus on products of fluxes and forces in the rate of entropy generation [9]. This phenomenological modification (i.e., replacing the magnitude of the velocity gradient tensor by the magnitude of the strain tensor) in viscoelastic solids is reasonable because anchorage-dependent cells dispersed homogeneously throughout porous biomaterials experience enhanced rates of proliferation in response to constant stress and harmonic stress. The stress-kinetic reciprocal (i.e., second) term on the right side of Eq. (4) represents a zeroth-order rate of nutrient consumption. Stress-free rates of nutrient consumption, given by the first term on the right side of Eq. (4), require the presence of nutrients, oxygen, and attached cells whose receptors form complexes with functional groups in the chemical structure of conformationally accessible proteins dispersed throughout, or embedded within, porous biomaterial matrices. Hence, the appropriate signaling exists for cells to consume nutrients and proliferate within the context of regenerative medicine. The form of Eq. (4) is sufficiently flexible to account for the effects of deformation that might change the reaction pathway or the products that are generated if another parallel pathway were equally important with comparable or lower activation energy [19,20] when external forces increase bond dissociation rate coefficients [25], relative to the stress-free kinetic contribution. In summary, scalar representations of tensorial quantities in the mechanochemistry of viscoelastic biomaterials have been respected in developing a self-consistent model for the coupling between time-dependent strain and reaction kinetics in stress-sensitive systems.

Stoichiometric requirements for nutrient consumption by anchorage-dependent mammalian cells

This application of tissue regeneration in viscoelastic biomaterials includes stress-free and stress-sensitive rates of nutrient consumption, where the latter is stimulated by harmonic excitation. It is necessary to connect the rate of nutrient consumption to the rate of cell proliferation. Effective biomass yields between 40% and 50% have been reported for a selected group of glucose-fed micro-organisms [21,22]. Hence, $\varepsilon_{cells}/\varepsilon_{nutrient} \approx 0.45$ is employed in Eq. (5), in consideration of the fact that some nutrient consumption could be channeled into other products and metabolic activities not related to cell proliferation, such as energetic support for cell mobility and sustainability. Yield coefficients that characterize cell mass produced per mass of oxygen consumed (i.e., $\varepsilon_{cells}/\varepsilon_{oxygen} \approx 0.45$) for the production of hematopoietic cells in 3-dimensional perfusion bioreactors suggest a 1:1 mass ratio for oxygen to nutrient consumption [23,24]. Both of these stoichiometric ratios (i.e., $\varepsilon_{cells}/\varepsilon_{nutrient} \approx 0.45$ and $\varepsilon_{oxygen}/\varepsilon_{nutrient} \approx 1$) are required to simulate tissue regeneration via the following relations between mass densities in porous biomaterials;

$$\rho_i(x,t) - \rho_i(x=L,t=0) = \upsilon_i \frac{\varepsilon_i}{\varepsilon_{nutrient}} \left\{ \rho_{nutrient}(x=L,t=0) - \rho_{nutrient}(x,t) \right\} \qquad (5)$$

where $\upsilon_{cells} = +1$, and $\upsilon_{oxygen} = -1$. These parameters are used in Eqs. (15) & (16).

Reaction-diffusion equation for one-dimensional diffusion and stress-sensitive consumption in biomaterials with rectangular symmetry

Fick's second law of diffusion with nutrient consumption (i.e., the *modified diffusion equation*) describes the transient and spatial dependence of the mass density of each reactive species (i.e., nutrients, oxygen, growth factors, etc.) within a viscoelastic biomaterial of thickness 2L that supports tissue regeneration [25-28]. For one-directional flux in the x-direction across the thinnest dimension of this matrix (i.e., transverse to the "stretch" direction), one must solve the reaction-diffusion equation for nutrient mass density, $\rho_{nutrient}(x,t)$;

$$\frac{\partial \rho_{nutrient}}{\partial t} = D_{A,effective,intra-tissue} \frac{\partial^2 \rho_{nutrient}}{\partial x^2} - R_{\substack{homogeneous \\ nutrient \\ consumption}} \qquad (6)$$

where time *t* accounts for transient response, $D_{A,effective,intra-tissue}$ is the effective diffusion coefficient for species A within porous viscoelastic biomaterials, and the total pseudo-homogeneous rate of nutrient consumption by anchorage-dependent cells within the matrix is calculated via Eq. (4) in the presence and absence of stress. Both sides of the regenerative matrix (i.e., $x = \pm L$) are exposed to a well-mixed nutrient medium at time, t=0. The required boundary conditions are;

$$\begin{aligned} &\rho_{nutrient} = \rho_{nutrient,medium};\ x = L;\ t > 0 \\ &\frac{\partial \rho_{nutrient}}{\partial x} = 0;\ \text{and}\ \rho_{nutrient} \Rightarrow 0;\ x = x_{critical}(t) \\ &\rho_{nutrient} = 0;\ t = 0;\ x_{critical} \le x < L \end{aligned} \qquad (7)$$

The zero-flux boundary condition at $x_{critical}$ is reminiscent of a boundary-layer problem because the central core is nutrient-starved at short times for all reasonable values of the intra-tissue Damköhler number. Dimensionless variables are introduced for nutrient mass density, spatial position in the thinnest dimension of the sample, and time;

Nutrient mass density; $\Psi_A = \dfrac{\rho_{nutrient}}{\rho_{nutrient,medium}}$

Spatial coordinate in thinnest dimension; $\eta = \dfrac{x}{L}$ (8)

Dimensionless diffusion time; $\tau = \dfrac{tD_{\mathrm{A,effective,intra-tissue}}}{L^2} = \dfrac{t}{\Theta_{Diffusion}}$

where $\Theta_{\mathrm{Diffusion}}$ represents a characteristic time constant for intra-tissue diffusion. This allows one to re-express the modified diffusion equation and its boundary conditions in dimensionless form for nutrient mass density $\Psi_{\mathrm{A}}(\eta,\tau)$;

$$\begin{aligned}
&\frac{\partial \Psi_A}{\partial \tau} = \frac{\partial^2 \Psi_A}{\partial \eta^2} - \Lambda^2_{A,stress-free}\Psi_A\Psi_{oxygen}\Psi_{cells} - \frac{\Lambda^2_{A,stress}}{1+De^2}\sqrt{\left\{\cos(\omega t) + De\sin(\omega t)\right\}^2} \\
&\Psi_A = 1;\ \eta = 1;\ \tau > 0 \\
&\frac{\partial \Psi_A}{\partial \eta} = 0;\ \text{and } \Psi_A \Rightarrow 0;\ \eta = 1 - \delta_{MTBLT}(\tau) \\
&\Psi_A = 0;\ \tau = 0;\ 1 - \delta_{MTBLT}(\tau) \le \eta < 1 \\
&De = \omega\lambda(T);\ \omega t = De\frac{\Theta_{Diffusion}}{\lambda(T)}\tau
\end{aligned} \qquad (9)$$

$\Lambda^2_{\mathrm{A,stress\text{-}free}}$ is the species specific stress-free intra-tissue Damköhler number that represents an order-of-magnitude ratio of the stress-free consumption rate to the rate of diffusion toward anchorage-dependent cells [9,25]. Hence:

$$\Lambda^2_{A,stress-free} = \frac{k_{stress-free}\rho^2_{nutrient,medium}L^2}{D_{\mathrm{A,effective,intra\text{-}tissue}}} \qquad (10)$$

where $\rho_{\mathrm{nutrient,medium}}$ is the mass density of nutrients in the vicinity of the external tissue surface, and $k_{\mathrm{stress\text{-}free}}$ is the pseudo-volumetric third-order kinetic rate constant for stress-free consumption. The stress-sensitive intra-tissue Damköhler number [5] is defined as follows:

$$\Lambda^2_{A,stress} = \frac{\kappa_{stress}\sigma_{AC}L^2}{\sqrt{2}E\rho_{nutrient,medium}D_{\mathrm{A,effective,intra\text{-}tissue}}} \qquad (11)$$

where κ_{stress} is the Onsager scalar coupling coefficient, σ_{AC} is the amplitude of harmonic stress excitation, and E is the viscoelastic biomaterial's static modulus of elasticity. Eq. (9) represents a rare example in the refereed journal literature where the Damköhler and Deborah numbers appear together in the reaction-diffusion equation to parameterize mass transfer in viscoelastic biomaterials subjected to stress. The Damköhler number (i.e., a

reaction-diffusion parameter) has been employed previously to model reaction and diffusion in cell cultures [25,29], microchannel bioreactors [30], and electrochemical biosensors immobilized within a highly dispersed mesh of carbon nanotubes [31]. The *pressure-sensitive* Damköhler number was developed recently to quantify mechano-sensitive zeroth-order bone tissue growth in response to centrifugal-force-induced hydrostatic pressure modulations in rotating-cup bioreactors [7]. The concept of the intra-tissue Damköhler number in biological systems is analogous to the intrapellet Damköhler number for heterogeneous catalysis in packed reactors [9,16]. Numerical solution of Eq. (9) via finite-difference calculus is awkward, due to zeroth-order stress-sensitive rates of nutrient consumption that must be extinguished in the tissue's central core at short times when nutrients have not diffused inward to a significant extent. There are very few literature references that invoke the von Kármán-Pohlhausen *profile* method and solve the modified diffusion equation with chemical reaction to predict transient mass transfer boundary layer thicknesses (i.e., 4 matches in Web of Science™ to *diffusion, reaction, von Kármán*). Profile methods have not been employed to solve mass transfer boundary layer problems in the presence of stress-sensitive biochemical kinetics [32].

Solution of the modified diffusion equation via the von Kármán-Pohlhausen integral method of boundary layer analysis

The transient reaction-diffusion equation, Eq. (9), was solved for dimensionless nutrient mass density, $\Psi_A(\varphi)$, and the dimensionless mass transfer boundary layer thickness $\delta_{MTBLT}(\tau;\Lambda_{A,stress\text{-}free},\Lambda_{A,stress})$ by postulating a quadratic function of the combined variable φ according to the von Kármán-Pohlhausen profile method of boundary layer analysis [28];

$$\Psi_A(\eta,\tau) = \Psi_A(\varphi) = \alpha + \beta\varphi + \zeta\varphi^2$$
$$\varphi = \frac{1-\eta}{\delta_{MTBLT}\left(\tau;\Lambda_{A,stress-free},\Lambda_{A,stress}\right)} \qquad (12)$$

The proposed quadratic function for dimensionless nutrient mass density Ψ_A in Eq. (12) is consistent with steady state profiles for zeroth-order rates of consumption in tissue with rectangular symmetry [9,28], for all values of both intra-tissue Damköhler numbers. Boundary conditions at η=1 [i.e., $\Psi_A(\varphi=0) = 1$] and η=1-δ_{MTBLT} [i.e., $\{\partial\Psi_A/\partial\eta\}_{\varphi=1} = \Psi_A(\varphi=1) = 0$] yield numerical values for the constants α, β, and ζ. Hence;

$$\Psi_A(\varphi=0) = \alpha = 1$$
$$\Psi_A(\varphi=1) = \alpha + \beta + \zeta = 0 \qquad (13)$$
$$\left\{\frac{\partial\Psi_A}{\partial\eta}\right\}_{\eta=1-\delta_{MTBLT}} = \frac{-1}{\delta_{MTBLT}\left(\tau;\Lambda_{A,stress-free},\Lambda_{A,stress}\right)}\left\{\frac{\partial\Psi_A}{\partial\varphi}\right\}_{\varphi=1} = \frac{-\beta-2\zeta}{\delta_{MTBLT}\left(\tau;\Lambda_{A,stress-free},\Lambda_{A,stress}\right)} = 0$$

with α=1, β=-2, ζ=1. If $\Psi_A \Rightarrow 0$ with zero slope at φ=1, then the initial condition is satisfied as $\varphi \Rightarrow \infty$. Upon substitution of the postulated profile for $\Psi_A(\varphi)$ via Eq. (12) into Eq. (9), multiplication by δ_{MTBLT}, and integration with respect to φ from 0 to 1, it is possible to obtain a first-order ordinary differential equation (ODE) for $\delta_{MTBLT}(\tau;\Lambda_{A,stress\text{-}free},\Lambda_{A,stress})$ that represents conservation of nutrient mass over the thickness of the boundary layer. This is illustrated in Eq. (14).

$$\left\{\frac{\partial \Psi_A}{\partial \tau}\right\}_\eta = \frac{d\Psi_A}{d\varphi}\left\{\frac{\partial \varphi}{\partial \delta_{MTBLT}}\right\}_\eta \frac{d\delta_{MTBLT}}{d\tau} = \frac{-\varphi}{\delta_{MTBLT}}\{\beta + 2\zeta\varphi\}\frac{d\delta_{MTBLT}}{d\tau}$$

$$-\frac{d\delta_{MTBLT}}{d\tau}\int_0^1 \varphi\{\beta + 2\zeta\varphi\}\,d\varphi = -\left\{\tfrac{1}{2}\beta + \tfrac{2}{3}\zeta\right\}\frac{d\delta_{MTBLT}}{d\tau} \qquad (14)$$

$$\left\{\frac{\partial^2 \Psi_A}{\partial \eta^2}\right\}_\tau = \frac{1}{\delta_{MTBLT}^2}\frac{d^2\Psi_A}{d\varphi^2} = \frac{2\zeta}{\delta_{MTBLT}^2}$$

$$\frac{1}{3}\frac{d\delta_{MTBLT}}{d\tau} = \frac{2}{\delta_{MTBLT}} - \frac{\delta_{MTBLT}\Lambda_{A,stress}^2}{1+De^2}\sqrt{\{\cos(\omega t) + De\sin(\omega t)\}^2} - \delta_{MTBLT}\Lambda_{A,stress-free}^2\int_{\varphi=0}^{1}\Psi_A(\varphi)\Psi_{oxygen}(\varphi)\Psi_{cells}(\varphi)d\varphi$$

The differential equation for δ_{MTBLT} in Eq. (14) reduces to Eq. (15) at steady state in the absence of stress (i.e., $\Lambda_{A,stress} \Rightarrow 0$) when the mass transfer boundary layer thickness is independent of dimensionless diffusion time τ.

$$\delta_{MTBLT}\left(\tau \Rightarrow \infty;\Lambda_{A,stress-free}\right) = \frac{\sqrt{2}}{\Lambda_{A,stress-free}\sqrt{\int_{\varphi=0}^{1}\Psi_A(\varphi)\Psi_{oxygen}(\varphi)\Psi_{cells}(\varphi)d\varphi}} \overset{\Psi_{cells}(\eta=1,\tau=0)=0.25}{\Rightarrow} \left\{\begin{array}{l} \overset{\Psi_{oxygen}(\eta=1,\tau=0)=0.75}{\Rightarrow} \dfrac{\sqrt{50}}{\Lambda_{A,stress-free}} \\ \overset{\Psi_{oxygen}(\eta=1,\tau=0)=1}{\Rightarrow} \dfrac{\sqrt{26}}{\Lambda_{A,stress-free}} \end{array}\right\} \qquad (15)$$

If the kinetics are zeroth-order instead of third-order, then the square-root term in the denominator of Eq. (15) is unity and the steady state dimensionless boundary layer asymptotically approaches a thickness of $\sqrt{(2)}/\Lambda_{A,stress\text{-}free}$ when the stress-free intra-tissue Damköhler number is greater than or equal to its critical value of $\sqrt{(2)}$ [9,28]. For complex stress-free nutrient consumption by anchorage-dependent cells requiring the presence of several ingredients for proliferation, porous biomaterials can operate further into the diffusion-limited regime at steady state such that the critical stress-free intra-tissue Damköhler number $\Lambda_{A,stress\text{-}free,critical}$ ranges from $\sqrt{(26)}$ to $\sqrt{(50)}$ [i.e., dependent upon $\Psi_{oxygen}(\eta=1,\tau=0)$ & $\Psi_{cells}(\eta=1,\tau=0)$] before regeneration ceases in the tissue's central core.

Stress-sensitive parametric analysis of mass transfer boundary layers when viscoelastic relaxation of the matrix occurs

The time-dependent ODE in Eq. (14) was solved for the development of $\delta_{MTBLT}(\tau;\Lambda_{A,stress\text{-}free},\Lambda_{A,stress})$, subject to the initial condition $\delta_{MTBLT}(\tau=0) = 0$ when the Deborah

number is on the order of unity, characteristic of viscoelastic relaxation. The initial rate of increase of δ_{MTBLT} with respect to τ is infinitely fast, according to the first term due to diffusion on the right side of the ODE in Eq. (16) that does not depend on the rates of stress-free or stress-sensitive consumption. Analogously, only the first term on the right side of Eq. (9) is important at τ=0, prior to the development of the mass transfer boundary layer. Previous analytical solutions of the modified diffusion equation with simple n^{th}-order kinetics (i.e., n=0,1,2) in biomaterials with rectangular symmetry [28] reveal that $\delta_{MTBLT} \approx 0.0346$ at $\tau = 10^{-4}$ when $\Lambda_{A,stress\text{-}free} = 4$ in the absence of stress. This pseudo-initial condition is employed in Eq. (16).

$$\frac{d\delta_{MTBLT}}{d\tau} = \frac{6}{\delta_{MTBLT}} - \frac{3\delta_{MTBLT}\Lambda^2_{A,stress}}{1+De^2}\sqrt{\{\cos(\omega t)+De\sin(\omega t)\}^2} - 3\delta_{MTBLT}\Lambda^2_{A,stress-free}\int_{\varphi=0}^{1}\Psi_A(\varphi)\Psi_{oxygen}(\varphi)\Psi_{cells}(\varphi)d\varphi$$

$$\Psi_A(\varphi) = \frac{\rho_{nutrient}}{\rho_{nutrient,medium}} = \alpha+\beta\varphi+\zeta\varphi^2$$

$$\Psi_i(\varphi) - \frac{\rho_i(x=L,t=0)}{\rho_{nutrient,medium}} = \upsilon_i\frac{\varepsilon_i}{\varepsilon_{nutrient}}\{1-\Psi_A(\varphi)\};\ i=cells,oxygen \qquad (16)$$

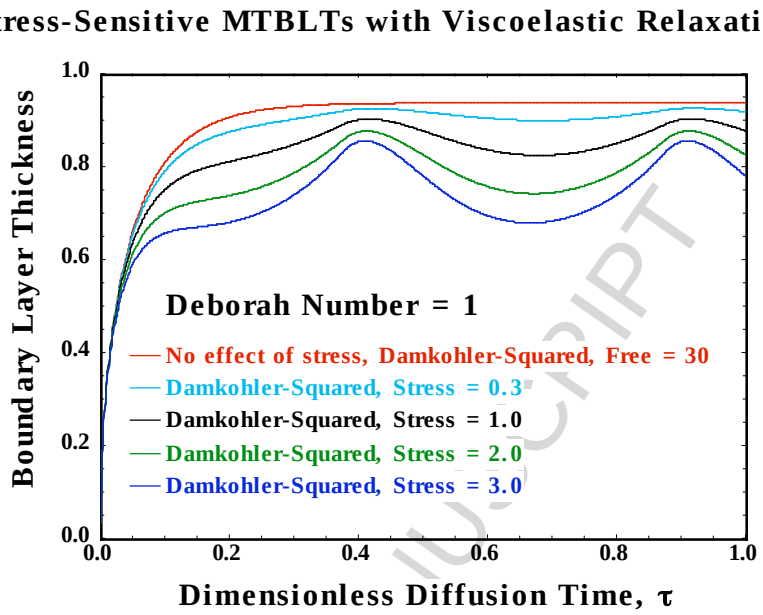
$$\int_{\varphi=0}^{1}\Psi_A(\varphi)\Psi_{oxygen}(\varphi)\Psi_{cells}(\varphi)d\varphi$$

$$\underset{\Psi_{cells}(\eta=1,\tau=0)=0.25}{\Rightarrow}\begin{cases}-0.0343+0.06\Psi_{oxygen}(\eta=1,\tau=0)-0.133\Psi_{cells}(\eta=1,\tau=0)+0.333\Psi_{oxygen}(\eta=1,\tau=0)\Psi_{cells}(\eta=1,\tau=0)\\ \approx 0.040\ \left[\text{when } \Psi_{oxygen}(\eta=1,\tau=0)=0.75\right];\ \text{or } \approx 0.076\ \left[\text{when } \Psi_{oxygen}(\eta=1,\tau=0)=1\right]\end{cases}$$

The external biomaterial surface at x=±L is exposed to dissolved oxygen in the well-mixed nutrient medium, and the entire porous matrix is seeded uniformly with attached cells. Time-dependent growth of the dimensionless mass transfer boundary layer $\delta_{MTBLT}(\tau)$, measured inward from the external tissue surface is illustrated in Figure#2 when viscoelastic relaxation occurs in porous biomaterials and the stress-free intra-tissue Damköhler number is slightly greater than its critical value, such that the tissue's inner core, defined by $0 \le \eta \le 1\text{-}\delta_{MTBLT}$, is starved of the essential ingredients required for cell proliferation.

Figure#2

von Kármán-Pohlhausen boundary layer predictions, based on the solution of Eq. (16) for nutrient diffusion and stress-sensitive consumption in porous biomaterials that experience viscoelastic relaxation (i.e., De = 1). The stress-free intra-tissue Damköhler number (i.e., $\Lambda^2_{A,stress\text{-}free} = 30$) is greater than its critical value of 26, according to Eq. (15), when the initial condition for cells and oxygen mass densities on the external biomaterial surface are: $\Psi_{cells}(\eta=1,\tau=0)=0.25$ and $\Psi_{oxygen}(\eta=1,\tau=0)=1$. The effect of stress on the nutrient mass transfer boundary layer increases from the stress-free uppermost curve to the lowermost curve. The critical stress-sensitive intra-tissue Damköhler number is approximately 5-7% of the stress-free intra-tissue Damköhler number. Parameters: $\omega = 2\pi$ radians/s, $\Theta_{diffusion}/\lambda = 2\pi$, 1000 steps in dimensionless diffusion time τ, from τ=0 to τ=1

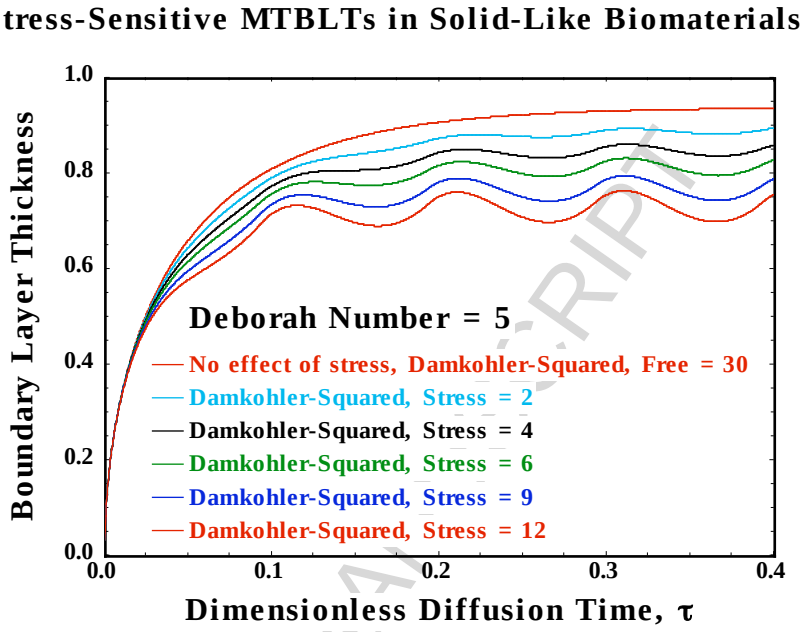


The amplitude of harmonic tensile stress excitation, σ_{AC}, represents a convenient parameter that allows one to systematically vary the stress-sensitive intra-tissue Damköhler number within the regime of linear viscoelastic response. Harmonic excitation of solid-like biomaterials at higher Deborah numbers in Figure#3 occurs at the same frequency (i.e., 1 hz.) relative to the simulations in Figure#2 when De=1, but the dimensional analysis of time in the oscillatory strain function includes the Deborah number [i.e., see Eq. (9)]. Hence, higher Deborah number response translates to more oscillations of the nutrient boundary layer thickness on the dimensionless time axis when the ratio of the diffusion time constant $\Theta_{diffusion}$ to the material response time $\lambda(T)$ remains the same, even though each time constant is longer in rigid solids relative to those that undergo viscoelastic relaxation. The effect of the Deborah number on dynamic compliance is primarily responsible for (i) smaller amplitude oscillatory response in Figure#3 relative to Figure#2, and (ii) the fact that larger stress-sensitive intra-tissue Damköhler numbers in rigid biomaterials are required to reduce the thickness of the nutrient boundary layer relative to the stress-free simulation.

Figure#3

von Kármán-Pohlhausen boundary layer predictions, based on the solution of Eq. (16) for nutrient diffusion and stress-sensitive consumption in solid-like biomaterials (i.e., De = 5). The stress-free intra-tissue Damköhler number (i.e., $\Lambda^2_{A,stress\text{-}free}$ = 30) is greater than its critical value of 26, according to Eq. (15), when the initial condition for cells and oxygen mass densities on the external biomaterial surface are: $\Psi_{cells}(\eta=1,\tau=0)=0.25$ and $\Psi_{oxygen}(\eta=1,\tau=0)=1$. The effect of stress on the nutrient mass transfer boundary layer increases from the stress-free uppermost curve to the lowermost curve. The critical stress-sensitive intra-tissue Damköhler number is approximately 20-

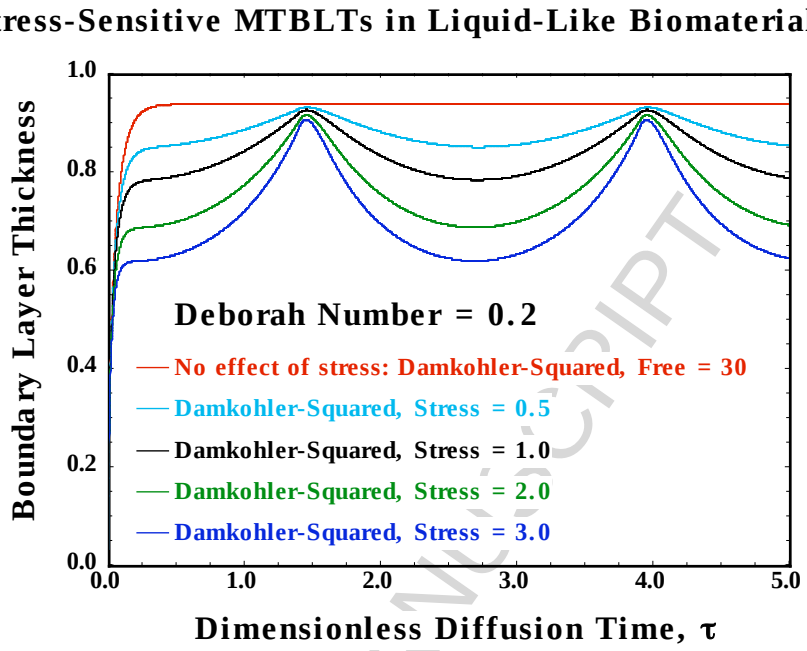
30% of the stress-free intra-tissue Damköhler number. Parameters: $\omega = 2\pi$ radians/s, $\Theta_{diffusion}/\lambda = 2\pi$, 1000 steps in dimensionless diffusion time τ, from τ=0 to τ=0.4



The critical value of the stress-sensitive intra-tissue Damköhler number, above which it is necessary to consider the effect of harmonic stress on nutrient consumption and tissue regeneration, is defined qualitatively as $\Lambda^2_{A,stress,critical}$ when the nutrient mass transfer boundary layer thickness decreases by ≈10% relative to the stress-free simulations in Figures 2-4. This reveals that $\Lambda^2_{A,stress,critical}$ is proportional to De, and corresponds to a larger fraction of the stress-free intra-tissue Damköhler number in rigid biomaterials characterized by higher Deborah numbers.

Figure#4

von Kármán-Pohlhausen boundary layer predictions, based on the solution of Eq. (16) for nutrient diffusion and stress-sensitive consumption in liquid-like biomaterials (i.e., De = 0.2). The stress-free intra-tissue Damköhler number (i.e., $\Lambda^2_{A,stress\text{-}free}$ = 30) is greater than its critical value of 26, according to Eq. (15), when the initial condition for cells and oxygen mass densities on the external biomaterial surface are: $\Psi_{cells}(\eta=1,\tau=0)=0.25$ and $\Psi_{oxygen}(\eta=1,\tau=0)=1$. The effect of stress on the nutrient mass transfer boundary layer increases from the stress-free uppermost curve to the lowermost curve. The critical stress-sensitive intra-tissue Damköhler number is approximately 3% of the stress-free intra-tissue Damköhler number. Parameters: $\omega = 2\pi$ radians/s, $\Theta_{diffusion}/\lambda = 2\pi$, 5000 steps in dimensionless diffusion time τ, from τ=0 to τ=5



Conclusions

Biological systems respond to stress, in general, via complex mechano-transduction pathways [33]. Some of the most favourable bioreactor designs for tissue regeneration are those based on (i) dynamic flow (i.e., bone, cartilage) [5,6], and cyclic stretching (i.e., tendon, ligament, bone). Tendons are stimulated by tension and bone cells proliferate at accelerated rates under compressive stress [7,34,35]. Recently, stress-sensitive kinetics have been identified experimentally in physicochemical systems that exhibit no biological influence [36]. The fields of *biorheology* and *mechanobiology* describe some aspects of stress-sensitive rates of nutrient consumption. The foundations of stress-kinetic scalar cross-phenomena are evident in the transport-phenomena-based rate of entropy generation and the corresponding *linear laws* proposed by Onsager, with assistance from Curie's theorem. It is desirable to develop regenerative tissue under reaction-diffusion conditions where the stress-free intra-tissue Damköhler number is less than its critical value to guarantee that the entire porous biomaterial matrix is exposed to nutrients, oxygen, and growth factors at steady state. The von Kármán-Pohlhausen integral method of boundary layer analysis of the reaction-diffusion equation reveals time-dependent growth of the mass transfer boundary layer inward from the external tissue/nutrient-medium interface toward the central core. Transient boundary layer predictions are compared in the presence and absence of harmonic stress excitation for viscoelastic biomaterials at large and small Deborah numbers. Thinner nutrient mass transfer boundary layers are stabilized at shorter dimensionless diffusion times when the stress-free intra-tissue Damköhler number increases above its critical value that depends on initial

conditions and stoichiometric parameters in the stress-free consumption rate. The critical stress-sensitive intra-tissue Damköhler number is proportional to the Deborah number, and corresponds to a larger fraction of the stress-free intra-tissue Damköhler number in rigid biomaterials characterized by higher Deborah numbers.

Acknowledgements

LA Belfiore gratefully acknowledges the Provincia Autonoma di Trento for research support during his extended sabbatical at the University of Trento. Professor Matt Kipper in the Department of Chemical and Biological Engineering at Colorado State University has been a source of inspirational support throughout all of these tissue-based bioreactor simulations. Michael Floren, a PhD student in the Department of Materials Engineering and Industrial Technologies at the University of Trento, is acknowledged for helpful discussions about cell proliferation in porous biomaterials.

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Nomenclature

$D_{A,effective}$	intra-tissue diffusion coefficient for species A
De	Deborah number; {material response time}/{time scale for deformation}
E	static modulus of elasticity of viscoelastic biomaterials
J_C	time-dependent creep compliance of viscoelastic biomaterials
J_D	distribution of viscoelastic relaxation times
J'	storage compliance; elastic contribution
J"	loss compliance; viscous contribution

$k_{stress\text{-}free}$ kinetic rate constant for 3rd-order pseudo-homogeneous stress-free rate of consumption; {volume/mass}^{2}/time

L one-half of the rectangular biomaterial's thickness in its thinnest dimension

$R_{homogeneous}$ rate of pseudo-homogeneous nutrient consumption, with contributions from stress-free and stress-sensitive kinetic pathways; mass/{volume-time}

t independent variable for transient response, time

T absolute temperature

x spatial coordinate measured in the thinnest dimension of the tissue

$x_{critical}$ critical value of the spatial coordinate in the thinnest dimension of rectangular tissue, below which reactants do not penetrate the central core of the tissue

Greek symbols

α,β,ζ coefficients in the quadratic function for dimensionless nutrient mass density Ψ_A, see Eq. (12)

γ time-dependent strain

δ_{MTBLT} time-dependent dimensionless mass transfer boundary layer thickness, measured inward from the external biomaterial surface

∇ gradient operator

$\varepsilon_{cells}/\varepsilon_{nutrient}$ stoichiometric ratio of the mass of cells produced per mass of nutrients consumed, ≈ 0.45

$\varepsilon_{oxygen}/\varepsilon_{nutrient}$ stoichiometric ratio of the mass of oxygen consumed per mass of nutrients consumed, ≈ 1

φ combined variable in the von Kármán-Pohlhausen quadratic molar density profile, see Eq. (12)

κ_{stress} scalar Onsager coefficient that couples deformation to the rate of consumption; mass/{volume-time}

$\lambda(T)$ material response time for viscoelastic biomaterials

$\Lambda_{A,stress\text{-}free}$ intra-tissue stress-free Damköhler number, which represents an order-of-magnitude estimate of the stress-free consumption rate with respect to the rate of species-specific diffusion toward the central tissue core

$\Lambda_{A,stress\text{-}free,critical}$ critical value of the intra-tissue stress-free Damköhler number, above which the tissue's central core is starved of essential nutrients at steady state

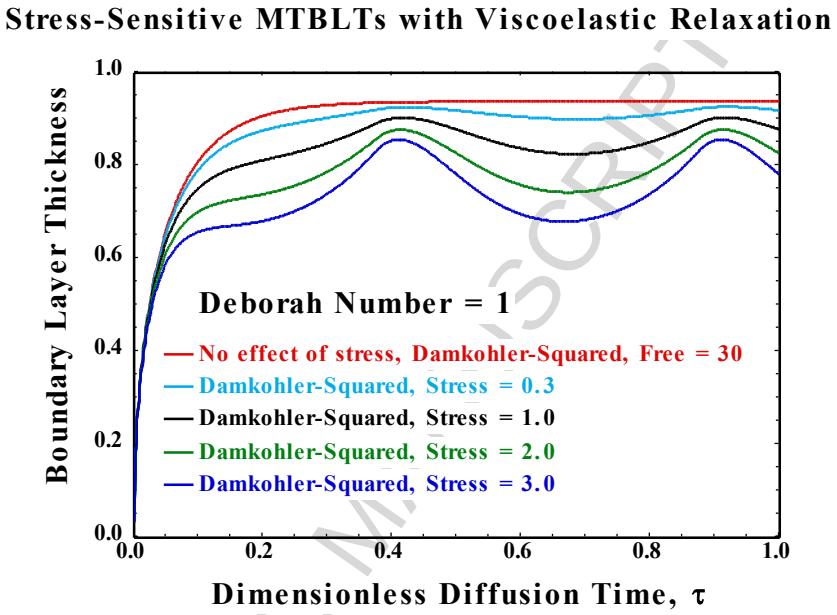
$\Lambda_{A,stress}$ intra-tissue stress-sensitive Damköhler number, which represents an order-of-magnitude estimate of the stress-dependent consumption rate with respect to the rate of species-specific diffusion toward the central tissue core

$\Lambda_{A,stress,critical}$ critical value of the intra-tissue stress-sensitive Damköhler number, above which it is necessary to consider the effect of harmonic stress on nutrient consumption and tissue regeneration, when the nutrient mass transfer boundary layer thickness decreases by ≈10% relative to stress-free simulations

η	dimensionless spatial coordinate in the thinnest dimension of tissue with rectangular symmetry; Eq. (8)
$\eta_{critical}$	critical value of the dimensionless spatial coordinate, below which reactants do not penetrate into the tissue's central core
υ_i	stoichiometric coefficients for reactants (i.e., oxygen) and products (i.e., cells)
ρ_{cells}	mass density of attached cells
$\rho_{nutrient}$	intra-tissue mass density of nutrients
$\rho_{nutrient,medium}$	mass density of nutrients on the external biomaterial surface
ρ_{oxygen}	intra-tissue mass density of dissolved oxygen
Ψ_A	dimensionless mass density of nutrients, defined in Eq. (8)
Ψ_{cells}	dimensionless mass density of attached cells
Ψ_{oxygen}	dimensionless mass density of dissolved oxygen
$\sigma(t,\omega)$	harmonic tensile stress excitation
σ_{AC}	amplitude of harmonic, time-dependent, stress excitation
σ_{DC}	time-independent stress excitation
$\sigma_{\Theta\Theta}$	normal tensile stress along the polar direction in spherical coordinates
$\Theta_{Diffusion}$	characteristic time constant for intra-tissue diffusion; $L^2/D_{A,effective,intra\text{-}tissue}$
τ	dimensionless independent time variable, defined in Eq. (8)
ω	frequency of harmonic, time-dependent, stress and strain

Stress-Sensitive Tissue Regeneration in Viscoelastic Biomaterials Subjected to Modulated Tensile Strain

Graphical Abstract



von Kármán-Pohlhausen boundary layer predictions for nutrient diffusion and stress-sensitive consumption in porous biomaterials that experience viscoelastic relaxation (i.e., De = 1)

Stress-Sensitive Tissue Regeneration in Viscoelastic Biomaterials Subjected to Modulated Tensile Strain

Research Highlights

- ***reaction-diffusion equation:*** a modification of Fick's second law is analyzed in porous biomaterial supports that contain anchorage-dependent cells subjected to harmonic stress
- ***critical intra-tissue Damköhler number:*** the rate of nutrient consumption relative to the rate of intra-tissue diffusion is quantified in the presence and absence of external stress
- ***Boltzmann superposition integral:*** is employed to calculate time-dependent strain in rigid and mobile viscoelastic biomaterials
- ***effect of viscoelastic relaxation on stress-sensitive kinetics:*** time-dependent strain is used as a metric to modify the rate of nutrient consumption in stress-sensitive systems
- von Kármán-Pohlhausen mass transfer boundary layer thickness is calculated to estimate the time required to achieve stable conditions for regenerative tissue growth