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**The Influence of Bioreactor Geometry and the Mechanical
Environment on Engineered Tissues**

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A three phase model for the growth of a tissue construct within a perfusion bioreactor is examined. The cell population (and attendant extracellular matrix), culture medium, and porous scaffold are treated as distinct phases. The bioreactor system is represented by a two-dimensional channel containing a cell-seeded rigid porous scaffold (tissue construct), which is perfused with a culture medium. Through the prescription of appropriate functional forms for cell proliferation and extracellular matrix deposition rates, the model is used to compare the influence of cell density-, pressure-, and culture medium shear stress-regulated growth on the composition of the engineered tissue. The governing equations are derived in O'Dea et al. "A Three Phase Model for Tissue Construct Growth in a Perfusion Bioreactor," Math. Med. Biol., in which the long-wavelength limit was exploited to aid analysis; here, finite element methods are used to construct two-dimensional solutions to the governing equations and to investigate thoroughly their behavior. Comparison of the total tissue yield and averaged pressures, velocities, and shear stress demonstrates that quantitative agreement between the two-dimensional and long-wavelength approximation solutions is obtained for channel aspect ratios of order 10^{-2} and that much of the qualitative behavior of the model is captured in the long-wavelength limit, even for relatively large channel aspect ratios. However, we demonstrate that in order to capture accurately the effect of mechanotransduction mechanisms on tissue construct growth, spatial effects in at least two dimensions must be included due to the inherent spatial variation of mechanical stimuli relevant to perfusion bioreactors, most notably, fluid shear stress, a feature not captured in the long-wavelength limit. [DOI: 10.1115/1.4001160]

1 Introduction

In vitro tissue engineering, the creation of replacement tissue from a sample of healthy cells or small explants, is an emerging field of enormous importance, with the potential to alleviate the chronic shortage of tissue available for implant [1]. A wealth of biological literature is available concerning the myriad complications involved in this process, from intracellular gene networks to tissue-level patterning and mechanics [2]; reviews are given in Refs. [1,3–5].

It is well known that replicating the in vivo environment is crucial to obtaining a healthy viable implant [6], especially where the resulting tissue is to be load-bearing, as with bone or cartilage; indeed, the importance of mechanical stimuli to tissue function is noted by many authors [7–9]. The process by which such stimuli are integrated into the cellular response is known as mechanotransduction. While many tissues share common mechanotransduction pathways, the influence of the mechanical environment will be cell-specific. For instance, fluid flow can have deleterious effects on cartilage regeneration, whereas fluid shear stress can enhance bone tissue formation [10–16]. Bespoke bioreactors are therefore required to provide appropriate physical (and biochemical) cues for different tissue engineering applications. In addition to enabling the provision of growth factors and other cell-

signaling molecules and aiding transfer of nutrients and waste products, many bioreactors are designed specifically with biomechanical effects in mind, providing stimulation via, for example, fluid shear stress, tensile or compressive forces applied on the macroscale, or via magnetic particles embedded in the cell membrane (see Refs. [17,18] for reviews).

The inherent complexity of the biomechanical and biochemical processes underlying tissue growth naturally lends itself to theoretical investigations. By constructing simplified mathematical representations of the in vitro system, such analyses aim to elucidate the dominant regulatory stimuli involved in tissue growth, to suggest explanations for observed tissue growth phenomena and to provide insights useful for the design of new bioreactor systems. Mathematical modeling of tissue growth has therefore received a great deal of attention; for example, much work has been dedicated to modeling tumor growth [19], angiogenesis [20–22], vasculogenesis [23], wound healing [24], as well as in vitro tissue engineering processes [3,4,25–27].

An experimental study of bone cell response to mechanical loading, in which a mechanical bioreactor was employed [28], provided the inspiration for this research. The bioreactor comprises a tissue construct within a culture medium-filled cylinder along which a flow is driven using a peristaltic pump (see Fig. 1). Cells seeded in a porous scaffold of 97% porosity are subjected to perfusion with a culture medium and direct compression using a piston. The experimental system has been employed to investigate the influence of perfusion and macroscale compression on tissue growth; in this theoretical study, we concentrate on the effect of perfusion and neglect the macroscale forcing provided by the piston.

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Fig. 1 The bioreactor system of El Haj et al. [28]

As already explained, the mechanical environment of the cells is known to profoundly affect tissue growth; the ease with which complex systems may be simulated to compute the behavior of a tissue construct in response to such considerations (for example, complex scaffold geometry or inhomogeneous mechanical properties) has led many to employ numerical techniques such as the finite element method (e.g., Kaasschieter et al. [29]). Considering a core of an underdeveloped tissue in a poroelastic model of a cartilage tissue, Kelly and Prendergast [30] showed that construct inhomogeneity dramatically reduces its mechanical integrity. Optimal design of porous scaffolds was discussed in Ref. [31] where the interplay between tissue growth and scaffold degradation, as well as the scaffold microstructure, was considered. Sanz-Herrera et al. [32] developed a multiscale model to investigate the interplay between scaffold design parameters and bone tissue regeneration. It was shown using finite element simulation that bone regeneration increases with scaffold stiffness and mean pore size; collapse was predicted for increased scaffold hydrolysis.

In the context of mechanotransduction-influenced growth (of bone cells), McGarry et al. [33] quantified the stresses experienced by individual adherent cells as a result of substrate strain and fluid shear stress. In contrast, Roose et al. [34] investigated the influence of growth-induced (residual) stresses on tumor cells within a macroscale multiphase framework in which the tumor was modeled as a growing poroelastic material. It was shown using this model and experimental evidence that tumor cell size is reduced by internal solid stress. Araujo and McElwain [35] also presented a general multiphase framework suitable for the consideration of such stresses. Employing a two-phase model, Byrne and Preziosi [36] considered the influence of the cells' environment on their proliferative rate in the context of tumor growth. The tumor was modeled as a viscous fluid phase interacting with an inviscid extracellular fluid. The proliferation of tumor cells was dependent on nutrient availability (governed by an advection-diffusion equation) and cell density, and a step function was used to switch between two different density and nutrient-dependent responses as the nutrient availability crossed a threshold value. The cells' response to growth-induced stress was modeled by introducing a parameter associated with such stress, and a critical stress level was predicted above which the tumor is eliminated. Chaplain et al. [37] presented a similar model considering tumor cells, normal cells, their associated extracellular matrices (ECMs), and a matrix-degrading enzyme.

In a recent study, O'Dea et al. [26] presented a three phase continuum model of tissue growth within the bioreactor system illustrated in Fig. 1. The bioreactor was represented as a two-dimensional channel containing a cell-seeded rigid porous scaffold, which is perfused with a culture medium. In common with many other studies of biological phenomena derived via a multiphase approach (e.g., Refs. [25,36,38,39] and references therein) the cells and associated ECM were represented as a viscous fluid phase that is distinct from the culture medium, and the porous scaffold was modeled as a rigid porous medium. Two factors of key importance to modeling the growth and adaptation of engineered tissue constructs were investigated: (i) cell-cell and cell-scaffold interactions, and (ii) the impact of mechanotransduction

mechanisms on construct morphology. The formulation was simplified via the long-wavelength limit (in which the bioreactor is assumed to be long and thin) and by considering constant, spatially homogeneous scaffold porosity. Numerical simulation of the model (validated by analytic solutions obtained in the limit of asymptotically small cell density) revealed that inclusion of cell-cell and cell-scaffold interactions leads to markedly different behaviors depending on the relative importance of cell aggregation and repulsion. It was further shown that cell density, pressure, and shear stress-mediated tissue growth generate qualitative differences in the composition of the resulting construct providing a means to identify the dominant regulatory stimuli in a cell population.

In Ref. [26] the limit of asymptotically small bioreactor aspect ratio was taken to simplify the governing equations and facilitate analytical progress. However, a distinguishing feature of the mechanical environment of tissue constructs in such bioreactors (and, indeed, in vivo) is the marked spatial variation (in the transverse and axial directions) of mechanical stimuli due, for instance, to scaffold and bioreactor geometry or inhomogeneous material properties; furthermore, certain methods of stimulating cells (e.g., via fluid shear stress) have inherent spatial variation. The long-wavelength limit, however, leads to a one-dimensional model in which transverse variations are neglected. Additionally, in many cases, the geometry of the bioreactor is such that the long-wavelength limit may not be appropriate (see Fig. 1). For these reasons, in this paper we extend the work of O'Dea et al. [26], employing the formulation presented therein and described above to investigate tissue growth in perfusion bioreactors with different aspect ratios. By exploiting finite element methods, we examine the behavior of the full system in the absence of simplifying limits and compare the results of our simulations to those produced by employing the long-wavelength limit for three different mechanotransduction regimes, establishing conditions under which the long-wavelength limit provides a good approximation to the full model and highlighting its limitations. The inclusion of two-dimensional geometry will provide detailed information of the spatial distribution of tissue within an engineered construct, together with the distribution of mechanical stimuli associated with certain loading regimes. Such information will prove invaluable in designing loading strategies for in vitro tissue engineering and may help pin-point areas of mechanical weakness within the construct, informing cell seeding or loading strategies for their elimination.

The remainder of the paper is organized as follows. In Sec. 2, the equations (together with appropriate boundary and initial conditions) employed to model the perfusion bioreactor are presented. In Sec. 3, these equations are solved numerically; uniform growth is considered in Sec. 3.1, while the influence of mechanotransduction-affected growth is studied in Sec. 3.2. Finally, in Sec. 3.3, the importance of two-dimensional bioreactor geometry is investigated by comparing the model simulations for various bioreactor aspect ratios to the behavior predicted in the long-wavelength limit. A discussion of the implications of our results for the field of tissue growth modeling is given in Sec. 4, together with directions for future research.

2 Model Formulation

The multiphase model employed in this paper is taken from Ref. [26] and builds on the general formulation given in Ref. [25]. In what follows it suffices to present the model equations, with a brief discussion of their provenance; the reader is directed to Refs. [26,25] for more details and, for example, Refs. [35,40,41], for detailed derivation and analysis of such models.

The growth of a tissue construct in a nutrient-rich perfusion bioreactor is considered, concentrating on the effect of cell-cell and cell-scaffold interactions and also that of mechanotransduction-regulated growth. We view the perfusion bioreactor as a two-dimensional channel containing a mixture of

Fig. 2 The two-dimensional domain Ω and associated boundary conditions. The arrows indicate the perfusion direction in the case $P_U > P_D$.

three interacting phases (a two-dimensional Cartesian geometry is chosen for simplicity; however, generalization to a cylindrical geometry is straightforward). The cells and ECM, which are modeled as a single phase (henceforth denoted the “cell phase”), and the culture medium are represented as viscous fluids; the scaffold is modeled as a spatially uniform porous phase, which is rigid and nondegradable. This simplifies the formulation so that we need only consider mass and momentum balances for the cell and culture medium phases (the scaffold phase enters the formulation via its volume fraction only). The fluid-based model of the growing cell phase avoids the generation of residual stress due to volumetric growth and mass conversion (modeling of tissue growth involving elastic deformation is beyond the scope of this study; examples of models including such effects are presented in Refs. [35,42]). Tissue growth is represented by an increase in the cell volume fraction, corresponding to the combined effects of cell proliferation and ECM deposition. Perfusion is represented by a pressure-driven flow of culture medium.

2.1 Model Equations and Constitutive Assumptions. The bioreactor is of length L^* and width h^* . The viscosities of the cell and culture medium phases are μ_n^* and μ_w^* , respectively, and the typical tissue growth timescale is denoted K^* . Asterisks distinguish dimensional quantities from their dimensionless equivalents.

We consider a Cartesian coordinate system $L^*x = L^*(x, y)$ and time K^*t , and the channel occupies the dimensionless region $0 \leq x \leq 1$, $0 \leq y \leq h = h^*/L^*$; see Fig. 2. The volume fractions of the cell, culture medium, and scaffold phases are denoted by θ_n , θ_w , and θ_s , respectively, while the dimensionless volume-averaged velocities, pressures, and stress tensors of the cell and culture medium phases are denoted $K^*L^*u_i = K^*L^*(u_i, v_i)$, $K^*\mu_n^*p_i$, and $K^*\mu_w^*\sigma^i$ (where $i=n, w$ denotes variables associated with the cell and culture medium, respectively). We assume that these dependent variables are functions of x and t .

The model is constructed by considering mass and momentum balances for each phase. As noted above, since the scaffold phase is assumed to be rigid and inert, we need not consider equations for this phase. Assuming that the fluid phases are incompressible with equal density and neglecting inertial effects, we obtain the following dimensionless mass and momentum conservation equations for the cell and culture medium phases, $i=n, w$:

$$\frac{\partial \theta_i}{\partial t} + \nabla \cdot (\theta_i \mathbf{u}_i) = S_i(\theta_k, p_k, \mathbf{u}_k) \quad (1)$$

$$\nabla \cdot (\theta_i \sigma^i) + \sum_{j \neq i} \mathbf{F}^{ij} = \mathbf{0} \quad (2)$$

where

$$\sigma^i = -p_i \mathbf{I} + \mu_i (\nabla \mathbf{u}_i + \nabla \mathbf{u}_i^T) - \frac{2}{3} (\nabla \cdot \mathbf{u}_i) \mathbf{I} \quad \text{for } i = n, w \quad (3)$$

$$\mathbf{F}^{ij} = (p_w + \psi_{ij})(\theta_j \nabla \theta_i - \theta_i \nabla \theta_j) + k \theta_i \theta_j (\mathbf{u}_j - \mathbf{u}_i) \quad \text{for } i, j = n, w, s \quad (4)$$

$$p_n = p_w + \Sigma_n + \theta_n \psi_{ns} \quad (5)$$

$$\Sigma_n = -\theta_n \nu + \frac{\delta_a \theta_n^2}{\theta_w} \quad \text{and} \quad \psi_{ns} = -\chi + \frac{\delta_b \theta_n}{\theta_w} \quad (6)$$

In Eq. (1) K^*S_i is the net material production term associated with phase i (mass conservation demands that $\Sigma S_i = 0$); in Eq. (2), $K^*\mu_w^*/L^*\mathbf{F}^{ij}$ is the interphase force exerted by phase j on phase i , obeying $\mathbf{F}^{ij} = -\mathbf{F}^{ji}$. These interphase forces comprise interphase viscous drag (with drag coefficient $\mu_w^*/L^{*2}k$) and active forces. The latter are embodied within the extra pressures $K^*\mu_n^*\Sigma_n$ and $K^*\mu_w^*\psi_{ns}$, arising due to cell-cell interactions and traction between the cell and scaffold phases, respectively; interactions between the culture medium and scaffold phases are assumed to involve only viscous drag ($\psi_{nw} = \psi_{ws} = 0$). Active forces contribute additional terms to the cell phase pressure in Eq. (5) and are defined in Eq. (6), in which $\nu, \chi, \delta_a, \delta_b > 0$ are dimensionless constants, controlling the cells' tendency to aggregate their affinity for the scaffold and cell-cell and cell-scaffold repulsion. Full details of the above choice of interphase interaction terms may be found in Ref. [25].

2.2 Mechanotransduction-Mediated Growth. In this study, we consider the influence of the local mechanical environment within a two-dimensional perfusion bioreactor on the growth and composition of a tissue construct. We model the cells' response to the following stimuli: contact inhibition caused by cell-cell interactions, stress caused by increases in tissue density, and the local fluid dynamics. These stimuli lead to either increased proliferation and ECM deposition or apoptosis, captured by postulating appropriate functional forms for the material transfer rates S_i . For simplicity we study these effects in isolation. The motivation for the following choices of material transfer is discussed in Refs. [26,27,36,37]; for concision we give only a short discussion of the modeling considerations embodied by these choices.

Contact inhibition and high stress levels inhibit cell proliferation, while a moderate level of stress appears to enhance tissue growth [7,34,37]. Roelofsen et al. [43] reported that excessively high hydrostatic pressure (>200 kPa) has an inhibitory effect on bone-specific gene expression; furthermore, many authors have demonstrated that exposure to intermittent hydrostatic compression inhibits bone resorption and stimulates bone formation [44]. Similarly, there is a wealth of literature pertaining to the sensitivity of bone cells to stimulation via flow-induced shear stress (e.g., Refs. [10,11,15,16]).

For each regulatory stimulus, we identify three distinct stages in the cell population's behavior: a quiescent phase (proliferation and ECM deposition are reduced), a proliferative phase (proliferation and ECM deposition are elevated), and an apoptotic phase. In each of these stages, the net tissue growth rate $\kappa(\phi)$ (defined by $S_n = \kappa(\phi)\theta_n$) takes a different value, where $\phi = \theta_n, p_n, \tau$ denotes density-, pressure-, and culture medium shear stress-mediated growth. The thresholds separating these stages are denoted ϕ_1 and ϕ_2 , where $0 < \phi_1 < \phi_2$. The culture medium flow-induced shear stress is defined as follows [45]:

$$\tau = \left(\left(\frac{\partial u_w}{\partial y} \right)^2 + \left(\frac{\partial v_w}{\partial x} \right)^2 \right)^{1/2} \quad (7)$$

For density- (representing contact inhibition-regulated growth) and pressure-dependent growth, a step function representation is employed such that the net growth rate takes the values $k_{1\phi}$ for $0 \leq \phi < \phi_1$, $k_{2\phi}$ for $\phi_1 \leq \phi \leq \phi_2$, and $-k_d$ for $\phi > \phi_2$. In contrast, to obtain a stable numerical solution, in the case of culture medium shear stress-mediated growth, the discontinuities in $\kappa(\tau)$ at τ_1 and τ_2 must be smoothed (see Refs. [26,46] for more details). For completeness, the functional forms employed for κ in the above regimes are specified as follows:

$$\kappa(\phi) = [k_{1\phi}H(\phi_1 - \phi) + k_{2\phi}H(\phi - \phi_1) - (k_{2\phi} + k_d)H(\phi - \phi_2)], \quad \phi = n, p_n \quad (8)$$

Fig. 3 The variation of the net cell proliferation rate $\kappa(\phi)$ with respect to the stimulus of interest, ϕ . (a) The step function form defined in Eq. (8) and (b) the smoothed form defined in Eq. (9). The dimensionless parameter k_1 represents the rate of cell proliferation, k_2 represents the combined rate of cell proliferation and ECM deposition, while k_d represents the combined rate of cell death and ECM degradation. The thresholds at which the cellular response changes are denoted ϕ_1 and ϕ_2 .

$$\kappa(\tau) = -\frac{k_1 - k_1\tau}{2}(\tanh(g(\tau - \tau_1)) - 1) - \frac{k_2 + k_d\tau}{2}(\tanh(g(\tau - \tau_2)) - 1) - k_d\tau \quad (9)$$

in which $H(\phi)$ is the Heaviside function. We recover a shear-dependent step function response in the limit $g \rightarrow \infty$. In Fig. 3 we present sketches of the net growth rates defined in Eqs. (8) and (9).

2.3 Boundary and Initial Conditions. The bioreactor described in Sec. 1 is represented by a two-dimensional channel Ω : $0 \leq x \leq 1$, $0 \leq y \leq h$ with boundary $\partial\Omega$. Appropriate conditions on the channel walls ($y=0, h$) are no-slip and no-penetration (of cells or culture medium). To model perfusion, a pressure-driven flow is imposed via up- and downstream pressures $K^*\mu_w^*P_U$ and $K^*\mu_w^*P_D$. This leads to normal stress conditions on each phase at $x=0, 1$ in which we assume that each phase bears its share of the stress in proportion to its volume fraction. We further assume that the flow up- and downstream is fully developed. These boundary conditions are illustrated in Fig. 2, in which $\hat{n}$ is the outward-pointing unit normal to the boundary $\partial\Omega$ of Ω .

The scaffold occupies a fixed region of the bioreactor and is defined as follows:

$$\theta_s(x, t) = \begin{cases} 0.03 & \text{for } a \leq x \leq b \\ 0 & \text{otherwise} \end{cases} \quad (10)$$

where $0 < a < b < 1$ are the edges of the scaffold.

Following Ref. [26], we consider the evolution of the tissue construct composition from the following initial state:

$$\theta_n(x, 0) = \frac{\hat{\theta}_n}{2} \left[\tanh\left(\frac{x - \alpha}{\varepsilon}\right) - \tanh\left(\frac{x - \beta}{\varepsilon}\right) \right] \quad (11)$$

where $\hat{\theta}_n$ is the maximum initial cell fraction, $x=\alpha$ and $x=\beta$ represent the left and right edges of the initial tissue construct, and ε governs the spatial gradient of θ_n near $x=\alpha, \beta$.

These conditions represent a small population of cells (of width $\beta-\alpha$) distributed within a scaffold (of width $b-a$) of uniform porosity (97%). The influence of alternative cell seeding strategies and a degrading scaffold phase represent natural modifications to

this work, which will be investigated in a subsequent publication. The values of these parameters used in this work are $a=0.25$, $b=0.75$, $\hat{\theta}_n=0.25$, $\alpha=0.4$, $\beta=0.6$, and $\varepsilon=0.02$.

3 Numerical Solution

In this section we present numerical solutions to Eqs. (1)–(6) subject to the boundary conditions given in Fig. 2 and the initial conditions (11) under a variety of different growth regimes. Specifically, we consider uniform growth as well as the mechanotransduction-mediated growth responses defined in Eqs. (7)–(9).

A diffusive term is included in Eq. (1) in order to convert it to a second order parabolic equation. While cells do exhibit random motion, we anticipate that the velocity field will be the dominant transport mechanism and diffusive terms will be negligible [47,48]. By including a diffusive term, we need not track explicitly the sharp interface, which is evident in the absence of diffusion. The inclusion of such artificial diffusion has a negligible effect on the solution [49] but requires additional boundary conditions on the cell volume fraction; we impose $\partial\theta_n/\partial n=0$ on $\partial\Omega$, where $\partial/\partial n$ denotes the derivative normal to $\partial\Omega$.

Solutions are obtained by decomposing the system into two components: (i) the velocity system, which is defined by momentum equations (2) (with $i=n, w$) and the statement of mass conservation for the multiphase mixture (Eq. (1) summed over all phases), and (ii) cell volume fraction equation (1). The former is solved at each timestep using a mixed-order finite element approximation, with piecewise-quadratic approximations for the velocities ($\mathbf{u}_n$ and $\mathbf{u}_w$) and a piecewise-linear approximation for the pressure (p_w) to ensure stability [50]. Equation (1) is solved by first using the method of characteristics to reformulate the equation as

$$\frac{d\theta_n}{dt} = S_n + D\nabla^2\theta_n - \theta_n \nabla \cdot \mathbf{u}_n \quad \text{on} \quad \frac{d\mathbf{x}}{dt} = \mathbf{u}_n \quad (12)$$

in which $d/dt = \partial/\partial t + \mathbf{u}_n \cdot \nabla$ represents a total derivative. An implicit Euler scheme coupled with a piecewise-quadratic finite element approximation is used to solve Eq. (12). Spatial and temporal discretizations are chosen to ensure convergence of the numerical solutions. Full details of the numerical algorithm employed (and details of convergence) are given in Ref. [46].

Table 1 Table of general dimensionless parameters in the model

Fixed parameters			
P_D	-	Downstream applied pressure	- $0.1/h^2$
P_U	-	Upstream applied pressure	- $0.3/h^2$
χ	-	Scaffold affinity parameter	- $0.05/h^2$
ν	-	Cell aggregation parameter	- $0.05/h^2$
δ_a	-	Intraphase repulsion parameter	- $0.05/h^2$
δ_b	-	Interphase repulsion parameter	- $0.05/h^2$
μ_n	-	Ratio of dimensional viscosities μ_n^*/μ_w^*	- 1.3
k	-	Interphase drag coefficient	- 10
a	-	Upstream boundary of scaffold	- 0.25
b	-	Downstream boundary of scaffold	- 0.75
D	-	Diffusion coefficient of cell volume fraction	- 0.001
Variable parameters			
h	-	Width of the reactor	- $h \in (0, 1]$

3.1 Uniform Growth. To illustrate the model behavior, we first consider the case of uniform growth, for which the rates of tissue growth and death are constant, choosing

$$S_n = -S_w = (k_m - k_d)\theta_n \quad (13)$$

wherein the dimensionless parameter k_m represents the combined rate of cell proliferation and ECM deposition, while k_d represents the combined rate of cell death and ECM degradation.

The parameter values employed in the numerical simulations are consistent with those used in Ref. [26], with the exception of the channel aspect ratio h and the viscous drag coefficient k , which is neglected in the long-wavelength model ($h \ll 1$) employed therein. In the absence of suitable experimental data, parameter values are selected to illustrate the behavior of the model for a given growth regime. The parameter values employed are summarized in Tables 1 and 2.

Table 2 Table of dimensionless parameters for each tissue growth regime

Constant proliferation: $S_n(\theta_n)$			
k_m	-	Rate of mitosis and ECM deposition	- 1.0
k_d	-	Rate of apoptosis	- 0.1
Cell density-dependent proliferation: $S_n(\theta_n)$			
θ_{n1}	-	Density threshold	- 0.4
θ_{n2}	-	Density threshold	- 0.6
k_{1n}	-	Rate of mitosis	- 0.8
k_{2n}	-	Rate of mitosis and ECM deposition	- 1.0
k_{dn}	-	Rate of apoptosis	- 0.1
Cell pressure-dependent proliferation: $S_n(\theta_n, p_n)$			
p_{n1}	-	Pressure threshold	- $0.15/h^2$
p_{n2}	-	Pressure threshold	- $0.25/h^2$
k_{1p}	-	Rate of mitosis	- 0.8
k_{2p}	-	Rate of mitosis and ECM deposition	- 1.0
k_{dp}	-	Rate of apoptosis	- 0.1
Culture medium shear stress-dependent proliferation: $S_n(\theta_n, \tau)$			
τ_{w1}	-	Stress threshold	- 0.1
τ_{w2}	-	Stress threshold	- 0.5
$k_{1\tau}$	-	Rate of mitosis	- 0.5
$k_{2\tau}$	-	Rate of mitosis and ECM deposition	- 1.0
$k_{d\tau}$	-	Rate of apoptosis	- 0.1

Typical results at a representative (dimensionless) time $t=1$ are presented in Fig. 4. The figures show how the cell volume fraction (θ_n), the pressures (p_n and p_w), the culture medium shear stress (τ), and the axial components of the velocities (u_n, u_w) evolve in response to the uniform growth regime. Pressures, stresses, and velocities display significant two-dimensional variation; this is especially marked in the case of the fluid flow and associated shear stress (see Figs. 4(d)–4(f)). Additionally, some transverse variation in construct composition is induced due to the culture medium flow (Fig. 4(a)).

In Secs. 3.2 and 3.3, we demonstrate the effect of coupling the growth of the tissue construct to the local mechanical environment (Sec. 3.2). In addition, the influence of the bioreactor aspect ratio on the predicted construct composition is investigated and comparison is made with the long-wavelength limit employed in Ref. [26] (Sec. 3.3).

3.2 Mechanotransduction. We now demonstrate the influence of mechanotransduction-regulated tissue growth by employing Eqs. (8) and (9), together with the parameter values given in Tables 1 and 2. Figures 5(a), 5(c), 6(a), and 6(c) contain illustrative plots of the cell volume fractions for the case $h=0.2$, revealing how the three different mechanotransduction stimuli affect the predicted construct composition.

Comparison of Figs. 5(a) and 5(c) illustrates the effect of the density-dependent tissue growth response. Cell proliferation and ECM deposition cease and apoptosis begins when the upper threshold θ_{n2} is reached; re-entry into the proliferative phase ensures that the resulting construct tends toward uniform density. In the pressure-dependent case (Fig. 6(a)) a different response is obtained since high pressure upstream and in the denser areas of the construct causes skewing in the downstream direction. (In Refs. [26,27] it was shown that in the absence of perfusion, regulation via cell density and pressure produces indistinguishable constructs. This result is reproduced in the current two-dimensional model; however, results are omitted for concision.) Lastly, Fig. 6(c) shows that regulation by shear stress causes a significant change in construct composition due to the marked spatial variation in shear stress throughout the channel. This becomes more pronounced as the width of the bioreactor is increased (results not included).

These simulations demonstrate that mechanical regulation of tissue growth leads to pronounced two-dimensional variation in construct composition in addition to that induced by perfusion with a culture medium (Fig. 4(a)). This is especially evident in the case of growth regulation by the local fluid dynamics where variation in the culture medium pressure (which influences p_n via Eq. (5)) and shear stress causes marked differences in construct composition. Density regulated growth results in a more uniform construct, with advection due to perfusion with a culture medium being the dominant mechanism inducing transverse variation. Figures 5(b), 5(d), 6(b), and 6(d) show corresponding results for reduced channel aspect ratio and exhibit qualitatively similar behavior. More details are given in Sec. 3.3.

3.3 Bioreactor Geometry. The numerical results presented above highlight the importance of including bioreactor geometry in at least two dimensions. In Ref. [26], the model formulation was simplified by employing the long-wavelength limit, in which the aspect ratio of the bioreactor is asymptotically small ($h \ll 1$). In this limit the viscosity associated with the rate of change in volume of each phase, as well as the interphase viscous drag terms, is neglected from the momentum equations (2). The pressure and the volume fraction of each phase are functions of x and t only and the flow is unidirectional at leading order; two-dimensional variation is therefore neglected. Below, we compare two-dimensional simulations for various channel aspect ratios to those obtained in the long-wavelength limit in order to determine the range of validity of the simplifying long-wavelength limit.

Fig. 4 Illustrative plots of (a) the cell volume fraction (θ_n), (b) the cell phase pressure (p_n), (c) the culture medium pressure (p_w), (d) the culture medium shear stress (τ), (e) the axial cell phase velocity (u_n), and (f) the axial culture medium velocity (u_w) in the regime of uniform cell proliferation, within a channel of dimension $[0,1] \times [0,0.1]$ at time $t=1$. The parameters are as described in Tables 1 and 2.

The simplified model is obtained by rescaling according to

$$y = h\bar{y}, \quad v_i = h\bar{v}_i, \quad p_w = \bar{p}_w/h^2, \quad (\Sigma_n, \psi_{ns}) = (\bar{\Sigma}_n, \bar{\psi}_{ns})/h^2,$$

$$\text{and } (P_U, P_D) = (\bar{P}_U, \bar{P}_D)/h^2 \quad (14)$$

The final two rescalings ensure that cell-cell and cell-scaffold interactions are retained at leading order and imply $(\nu, \delta_a, \chi, \delta_b) = (\bar{\nu}, \bar{\delta}_a, \bar{\chi}, \bar{\delta}_b)/h^2$ (see Table 1); the remaining parameters are $\mathcal{O}(1)$. Averaging across the channel in the transverse direction, it is straightforward to obtain expressions for the averaged axial velocities $\langle u_w \rangle$ and $\langle u_n \rangle$, substitution of which into the (averaged) mass conservation equations yields (dropping the overbars)

$$\frac{\partial \theta_n}{\partial t} - \frac{\partial}{\partial x} \{ (\Theta - \theta_n) \langle u_w \rangle \} = S_n + D \frac{\partial^2 \theta_n}{\partial x^2}, \quad \langle u_w \rangle = -\frac{1}{12} \frac{\partial p_w}{\partial x} \quad (15)$$

$$\frac{\partial}{\partial x} \left\{ \left(\frac{\theta_n}{\mu_n} + (\Theta - \theta_n) \right) \frac{\partial p_w}{\partial x} \right\} + \frac{1}{\mu_n} \frac{\partial}{\partial x} \left\{ \left(\frac{\partial (\theta_n \Sigma_n)}{\partial x} + \theta_n \theta_s \frac{\partial \psi_{ns}}{\partial x} \right) \right\} = 0 \quad (16)$$

and the boundary conditions (Sec. 2.3) become

$$p_w = P_U \quad \text{at } x=0 \quad (17)$$

$$p_w = P_D \quad \text{at } x=1 \quad (18)$$

$$\frac{\partial \theta_n}{\partial x} = 0 \quad \text{at } x=0 \quad \text{and } x=1 \quad (19)$$

In Eqs. (15) and (16), $\Theta = 1 - \theta_s$ denotes the scaffold porosity and μ_n is the relative viscosity of the cell and culture medium phases. Initial conditions (11) are applied (with $y=0$).

Equations (15) and (16) are solved in a manner analogous to the two-dimensional case. Equation (16) is solved for p_w using θ_n

Fig. 5 Illustrative plots of the cell volume fraction (θ_n) for ((a) and (b)) constant cell proliferation and ((c) and (d)) cell density-dependent proliferation in a channel of widths $h=0.2$ and $h=0.01$ at $t=1$. The parameter values are given in Tables 1 and 2.

from the previous timestep (with a linear finite element approximation); a solution to Eq. (15) is obtained by applying the method of characteristics and the implicit Euler method along with a linear finite element approximation for θ_n , iterating between the solutions of each equation. In our earlier work [26], numerical solution of Eqs. (15) and (16) was obtained via finite difference method; excellent agreement between the two schemes is found (results omitted).

Solutions to Eqs. (1)–(6), with bioreactors of widths $h=0.2$ and 0.01 , for all four growth regimes are presented in Figs. 5 and 6. It is clear that, for all growth regimes, as h is decreased (to $h=0.01$), the transverse variation of the cell volume fraction is significantly reduced. This notwithstanding, in each growth regime, a qualitatively similar response to the chosen growth regime is observed for large and small channel aspect ratios.

Figure 7 shows how the solutions to Eqs. (1)–(6) (averaged across the bioreactor in the y -direction) in the regime of uniform growth compare with the long-wavelength approximation for the choices $h=0.2$, 0.1 , and 0.01 . Figure 8 shows the averaged cell volume fraction obtained under cell density-, cell pressure-, and fluid shear stress-regulated growth. The remaining variables are omitted for brevity. For all growth regimes the long-wavelength approximation is in qualitative agreement with the averaged two-dimensional simulations, even for relatively large aspect ratio, and this agreement improves as the aspect ratio is reduced. However, as Figs. 7(b) and 7(d) reveal, the qualitative agreement of those variables associated with the cells' mechanical environment is lost for $h \geq 0.2$.

Quantitative comparison between the results of the two-dimensional simulations and the long-wavelength limit is made via the transient cell yield defined by

$$\bar{\theta}_n(t) = \int_{\Omega} \theta_n(\mathbf{x}, t) dV \quad (20)$$

and the L_2 norm of the deviation between the two-dimensional and long-wavelength solutions $\|\theta_n^{2D} - \theta_n^{LW}\|_{L_2}$. The superscripts 2D and LW denote the two-dimensional simulations and the long-wavelength approximation (extended across the two-dimensional domain), respectively.

The transient yield for bioreactors of width $h=0.2, 0.1, 0.01$ and the long-wavelength limit, in each of the growth regimes examined above, is depicted in Fig. 9. The difference in transient yield is small for all cases. However, the yield, being a measure of total cell density, does not take the spatial distribution of cells into account. In order to investigate the spatial distribution of the construct we use the L_2 norm.

Figure 10 depicts the L_2 norm of the disparity between the two-dimensional and long-wavelength limit solutions for bioreactors of widths $h=0.2, 0.1, 0.01$ in each of the growth regimes examined above. In each case the long-wavelength approximation improves as the aspect ratio decreases. For uniform proliferation or cell density- and pressure-mediated tissue growth, excellent agreement is displayed for $h=0.01$ and the L_2 norm remains approximately constant over time. Figure 10(d) shows that in the case of tissue growth governed by the culture medium shear stress the error grows as the tissue density increases and that there are significant differences between the predicted construct composition in the long-wavelength and two-dimensional models. This is due to the marked spatial variation in culture medium shear stress across the bioreactor (seen in Fig. 4(d)), which is not captured by the long-wavelength limit.

Fig. 6 Illustrative plots of the cell volume fraction (θ_n) for ((a) and (b)) cell pressure-dependent proliferation and ((c) and (d)) culture medium shear stress-dependent proliferation in a channel of widths $h=0.2$ and $h=0.01$ at $t=1$. The parameter values are given in Tables 1 and 2.

Fig. 7 Plots comparing the averaged (a) cell volume fraction ($\langle \theta_n \rangle$), (b) cell phase pressure ($\langle p_n \rangle$), (c) weighted axial cell phase velocity ($\langle \theta_n u_n \rangle$), and (d) culture medium shear stress ($\langle \tau \rangle$) at $t=1$ for uniform growth in a channel of dimensions of $(0,1) \times (0,h)$ for $h=0.2$ (---), $h=0.1$ (-.-), $h=0.01$ (—), and the long-wavelength limit (···). The remaining parameter values are given in Tables 1 and 2.

Fig. 8 Comparison of the averaged cell volume fraction ($\langle \theta_n \rangle$) for (a) cell density-dependent proliferation, (b) cell pressure-dependent proliferation, and (c) culture medium shear stress-dependent proliferation at $t=1$ in a channel of dimensions of $(0,1) \times (0,h)$, for $h=0.2$ (---), $h=0.1$ (-.-), $h=0.01$ (—), and the long-wavelength limit (···). The remaining parameters are given in Tables 1 and 2.

The above comparisons show that the long-wavelength limit provides a good approximation to the averaged cell volume fraction and weighted axial cell phase velocity when compared with the results of the full two-dimensional model. Furthermore, the transient yield obtained in each of the growth regimes is well captured by the long-wavelength predictions. In all cases, the long-wavelength prediction becomes more accurate as the bioreactor aspect ratio decreases. Thus, if the aim of the modeling is to predict average cell number and how it varies with time, the predictions obtained in the long-wavelength limit provide a good approximation. However, although aiding analysis, this simplifying limit neglects the two-dimensional variation in construct composition. Figures 5(c), 6(a), and 6(c) demonstrate that due to the variation in the cells' mechanical environment throughout the channel, the composition of the construct varies significantly in the transverse direction; indeed, Figs. 4(a) and 5(a) demonstrate that some transverse variation is obtained even in the case of uniform or cell density-dependent growth. Advection of cells with the culture medium is the dominant mechanism giving rise to this. The two-dimensional spatial variation in the cells' mechanical environment leads to significant qualitative differences between the predicted averaged cell pressure and culture medium shear stress between the two-dimensional ($h=0.2$) and long-wavelength predictions (see Figs. 7(b) and 7(d)). The L_2 norms presented in Fig. 10 further reveal that spatial variations are significant in all growth regimes for $h=0.2, 0.1$ and, for shear stress-mediated growth, even for $h=0.01$.

4 Discussion

In this paper, we have examined a multiphase model of tissue growth within a perfusion bioreactor. The bioreactor was modeled as a two-dimensional channel containing a mixture of three separate phases, which represent the cells (together with the associated ECM), a rigid porous scaffold, and a culture medium. The model employed was based on that presented in Ref. [26] (which ex-

ploited the general multiphase formulation given in Ref. [25]) in which the cell and culture medium phases were modeled as viscous fluids; the rigid scaffold phase was assumed to be spatially homogeneous and inert.

In Ref. [26], the long-wavelength limit (in which the bioreactor aspect ratio is assumed to be asymptotically small) was employed to simplify the model equations and make analytical progress. However, a distinguishing feature of the cells' mechanical environment in such in vitro tissue engineering systems (or, indeed, in vivo) is the marked spatial variation of mechanical stimuli; furthermore, bespoke bioreactors may have large aspect ratios for which the long-wavelength limit is not valid.

To determine the importance of such effects, in this study we have provided numerical solutions to the full two-dimensional equations, investigating the growth of tissue constructs within bioreactors with different aspect ratios under the influence of a range of biologically motivated mechanotransduction mechanisms. Our results demonstrate the spatial variation of mechanical stimuli relevant to tissue growth within a perfusion bioreactor, especially those associated with culture medium flow, and their influence on eventual tissue construct composition. The significant differences in predicted construct composition resulting from regulation by different mechanical stimuli raise the possibility of employing models of this type in tandem with appropriate experimental data to identify the dominant regulatory stimuli in a cell population (as concluded in Refs. [26,27]); we remark here, however, that the robustness of these conclusions should be tested by the extension of the model to accommodate nutrient-limited growth since the delivery of nutrients to downstream regions of the construct may become growth rate-limiting after many days in culture (studies incorporating nutrient-limited growth include Refs. [51–53]).

Our results indicate that the long-wavelength limit captures much of the qualitative behavior of the model when considering average cell yields, even for relatively large aspect ratios. To as-

Fig. 9 Comparison of transient cell yields over time for bioreactors of widths: $h = 0.2$ (---), $h = 0.1$ (- - -), $h = 0.01$ (—), and the long-wavelength limit (···) for different mechanotransduction regimes. The parameter values are given in Tables 1 and 2. (a) Uniform proliferation, (b) cell density-dependent growth, (c) cell pressure-dependent growth, and (d) culture medium shear stress-dependent growth. The arrows show the direction of decreasing aspect ratio h .

sess the significance of two-dimensional spatial effects in the predicted construct composition compared with the results obtained in the long-wavelength limit, the L_2 norm was evaluated. For aspect ratios $h = 0.2, 0.1$, spatial variations were shown to be significant for all growth regimes; agreement between the two-dimensional and long-wavelength models improved with decreasing h . However, for shear stress-mediated growth, the predicted construct composition showed significant qualitative differences compared with the long-wavelength predictions even for $h = \mathcal{O}(10^{-2})$.

We conclude that the decision to employ the long-wavelength limit to simplify the full two-dimensional model should be motivated by the available data. The long-wavelength limit provides a good approximation to the averaged tissue yield, even for relatively large aspect ratio bioreactors. This limit therefore provides a useful model of tissue growth, allowing simplified investigation of such processes within a formulation, which encompasses many considerations important to tissue growth modeling; its simplicity in comparison to the full two-dimensional model makes it a natural framework with which to use crude experimental data (i.e., total cell number) to fit the model parameters for specific bioreactor systems. On provision of detailed histological data of the tissue construct composition, these parameter estimates may then be employed in the full two-dimensional model, allowing accurate model comparisons of the tissue distribution within the scaffold, as well as providing detailed information regarding the spatial distribution of mechanical stimuli. Such information is invaluable in designing loading strategies for in vitro tissue engineering. Furthermore, inferences may be drawn regarding areas of mechanical weakness within the construct: different seeding strategies coupled with knowledge of the tissue growth response to different culture conditions have the potential to eliminate such defects.

Investigation into the effect of such seeding strategies on tissue construct growth will be presented in a subsequent study.

The formulation employed in this study naturally lends itself to tissue growth modeling, capturing the interactions between the numerous constituent phases involved. For simplicity, we have restricted attention to a rigid, nondegrading scaffold, the remaining phases being modeled as viscous fluids and so our model applies to those constructs whose solid characteristics are dominated by scaffold rigidity. We note that our formulation is versatile, and elastic or viscoelastic constitutive modeling assumptions for these phases may be accommodated. Lastly, we remark that the modeling of mechanotransduction-mediated growth employed highly idealized functional forms, with each stimulus acting in isolation; in reality, these phenomena are likely to interact in a complex way to produce the cells' overall response. Additionally, conclusions relevant to specific tissue engineering systems may in principle be obtained by modifying the mechanotransduction responses in line with appropriate experimental data; however, such modifications are beyond the scope of this paper. Our simplified representation allows clear illustration of the importance of such effects within a tissue growth framework, and a further publication will consider competing growth stimuli as well as the interplay between scaffold degradation and nascent tissue growth.

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Fig. 10 The L_2 norm $\|\theta_n^{2D} - \theta_n^{LW}\|_{L_2}$ over time for $h=0.2$ (· · · · ·), $h=0.1$ (— — —), and $h=0.01$ (—), for different mechanotransduction regimes. The parameter values are given in Tables 1 and 2. (a) Uniform proliferation, (b) cell density-dependent growth, (c) cell pressure-dependent growth, and (d) culture medium shear stress-dependent growth. The arrows show the direction of decreasing aspect ratio h .

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Nomenclature

- $\theta_n, \theta_w, \theta_s, \Theta$ = volume fraction of cell, culture medium, and scaffold phases; porosity
- $p_w^*, p_n^*, \Sigma_n^*, \psi_n^*$ = culture medium and cell phase pressure; intraphase pressure and cell-scaffold tractions (N m^{-2})
- S_n^*, S_w^* = net material transfer rate into cell and culture medium phases (s^{-1})
- σ_n^*, σ_w^* = stress tensor for the cell and culture medium phases (N m^{-2})
- u_n^*, u_w^* = velocity of cell and culture medium phases (ms^{-1})
- F^{*wn}, F^{*nw} = forces (per unit volume) between cell and culture medium phases (N m^{-3})
- $\mathbf{x}^* = (x^*, y^*)$ = spatial coordinate in horizontal and vertical directions (m)
- t^* = time (s)

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