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Tensegrity as a main determinant of tissue morphogenesis: Mathematical modelling of cell interactions with collagen and matrigel.

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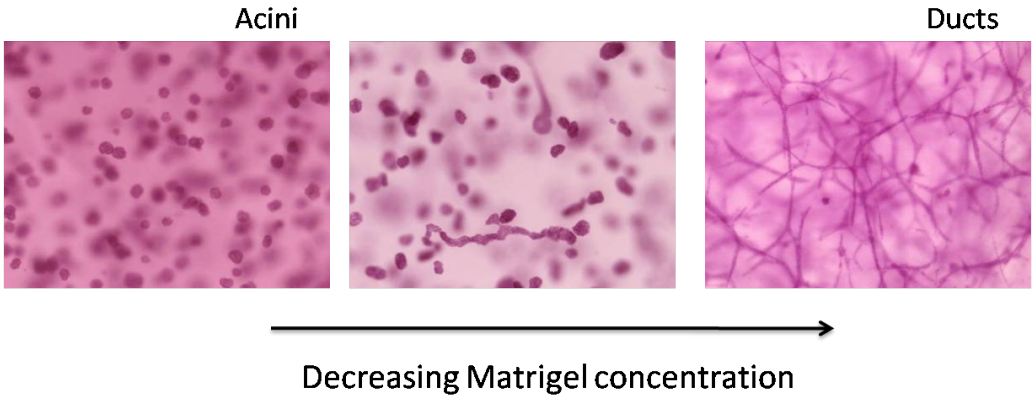
1 Introduction

In the late 19th century, embryologists explained developmental events in terms of mechanics [22]. This tradition was set aside with the emergence of biochemistry and molecular biology. However, these latter disciplines have not yet provided explanations of how the organism is built; hence there is a renewed interest in the role of physical force as a determinant of biological structures. The integrity of tissues is maintained by a synergy between balanced tension and compression components (tensegrity). From the macro-scale to the nano-scale, the organism is shaped by tensional forces (balanced push and pull) [12]. Although considerable progress has been achieved in this field during the last two decades, a detailed analysis of these physical force-mediated processes is difficult to achieve in the embryo. Tissue engineering is providing experimental models to facilitate these studies [13]. For example, Engler *et al* have shown that the rigidity of the intercellular matrix can determine the commitment fate of stem cells [8].

A novel 3D tissue organogenesis model of the mammary gland has been developed by the experimental groups of Soto and Sonnenschein [16]. Epithelial cells are seeded into a substrate containing an anisotropic collagen gel, which contains fibrous collagen strands embedded within a matrix, mixed with varying quantities of matrigel, nutrient solution and water (these last three behave isotropically, and will be collectively called "medium"), either with or without fibroblast cells. The fibroblasts perform two main functions, rearranging the fibrous matrix and extruding new collagen fibres. The assay can be used to assess the roles played by different components present in the assay on the formation of the mammary gland. The epithelial cells undergo cell proliferation and organise themselves into small aggregates, forming either acini (spherical structures) or ducts (elongated cylindrical structures). The proportions in which these structures form is known to depend on the biomechanical properties of the substrate, and how it evolves over time. As the concentration of the matrigel decreases (*i.e.* the viscosity of the medium decreases) the aggregates formed alter from predominantly acini to predominantly ducts (see Figure 1). A similar effect is observed when fibroblasts are added to the culture.



Models of cell-ECM interactions tend to treat the ECM as an isotropic elastic or viscoelastic material [14, 19, 23, 24]. However, ECM often displays evolving anisotropic properties (*e.g.* through the presence of the anisotropic collagen gel described above), and these can have a large effect on the system; the feedback between mechanical effects of the ECM on the cells, and conversely the cells on the ECM is beautifully shown in video 6 of [25]. Models which do account for this anisotropy [2, 5], incorporating a fibrous network and an interstitial fluid, are too complicated to be analytically tractable. A selection of simplifying assumptions have been made

in the literature to make the problem tractable. The majority of models which include remodelling of the ECM (*e.g.* [4]) have degradation of the ECM density by cell movement (some including anisotropic effects due to the presence of the fibres) but no evolution of the fibre angle. Olsen *et al.* [18] do consider realignment of fibres by the cells, however they only allow fibres to be aligned in one of two perpendicular directions; the realignment switches the fibre from one state to the other.

During the study group, we developed a simple mathematical model to describe the effects of matrix anisotropy on the architecture of cellular aggregates. This generic problem was considered, in part due to the absence of experimental data on the mechanical properties of the matrix and because it illustrates the role of mechanics in biological pattern formation: to date there have been very few studies that take into account the anisotropic nature of fibrous materials such as collagen, even though they are ubiquitous *in vivo*. The remainder of this report is structures as follows. In §2 we formulate a multiphase model including the anisotropic effects of the collagen. This model is analysed for sparsely-seeded cells in §3. We give our conclusions and discuss further avenues to explore in §4.

2 Model formulation

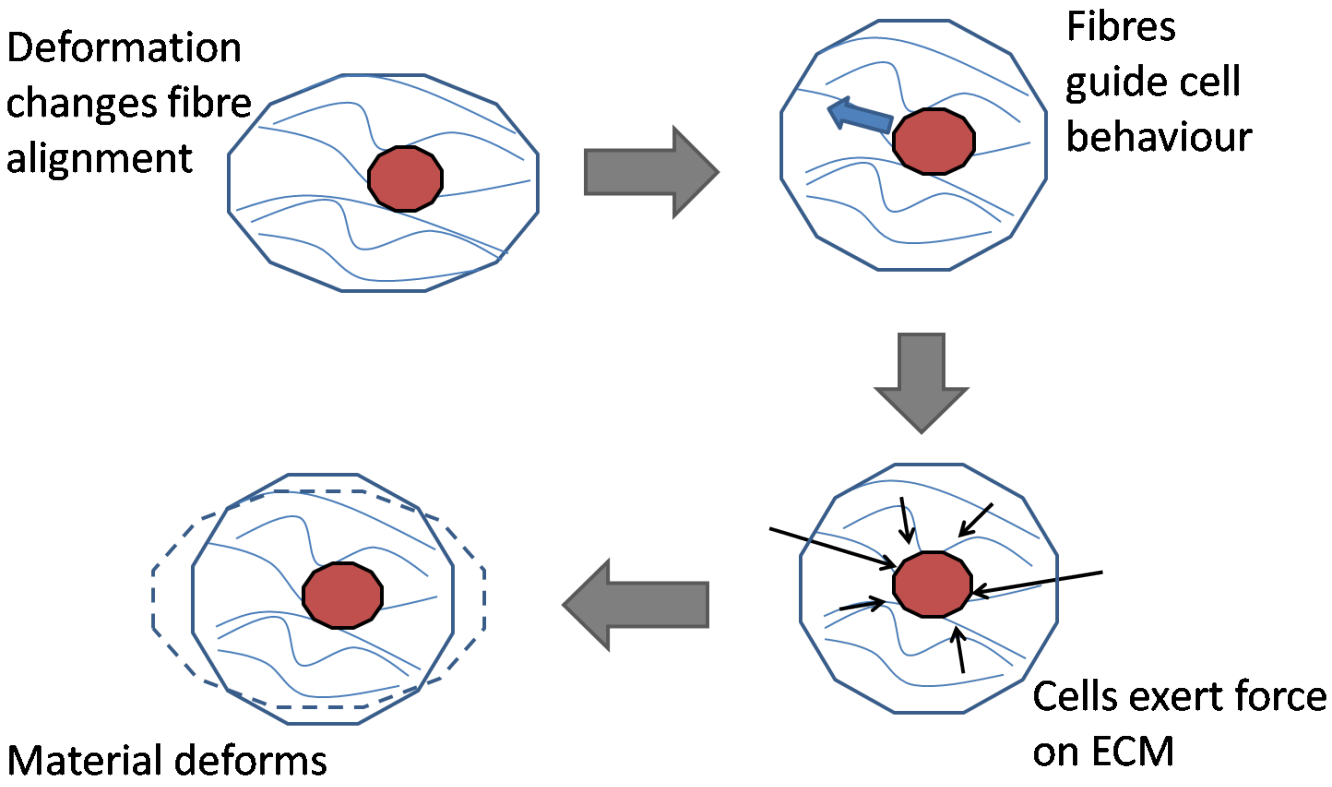


Figure 1: A sketch of the model structure.

A simple schematic of the model structure is given in figure 1. Cells within the material move according to the fibre alignment, and exert forces. Fibres within the matrix transmit these forces, affecting the deformation of the material. This alters the fibre alignment, which in turn guides the cell migration. This feedback between cells and fibres is the essence of the model which we describe below.

2.1 Governing equations

We treat the tissue as a three phase mixture, comprising cells, collagen and a combination of matrigel and culture medium (which we hereafter refer to simply as 'matrigel' or 'culture medium'). For simplicity we neglect the fibroblasts and their effect on the anisotropy; structures are still observed to form (albeit more slowly) in the absence of these cells and neglecting them significantly simplifies the problem. The volume fractions of the three phases are denoted ϕ_n, ϕ_c and ϕ_m respectively. We assume there are no voids, so

$$\phi_n + \phi_c + \phi_n = 1. \tag{1}$$

We assume that cells, collagen and matrigel have the same constant density and we can thus exclude this factor from the relevant mass balance equations. We assume further that increases in cell mass due to cell proliferation causes a corresponding reduction in the mass of culture medium, and that this exchange takes place at rate $S\phi_n\phi_m$. Collagen, however, is assumed to be conserved. We denote the velocities of the cells, collagen and matrigel by $\boldsymbol{v}_n$, $\boldsymbol{v}_c$ and $\boldsymbol{v}_m$. Conservation of mass then supplies

$$\frac{\partial \phi_n}{\partial t} + \nabla \cdot (\phi_n \boldsymbol{v}_n) = S\phi_n\phi_m, \tag{2a}$$

$$\frac{\partial \phi_c}{\partial t} + \nabla \cdot (\phi_c \boldsymbol{v}_c) = 0, \tag{2b}$$

$$\frac{\partial \phi_m}{\partial t} + \nabla \cdot (\phi_m \boldsymbol{v}_m) = -S\phi_n\phi_m. \tag{2c}$$

We denote by $\boldsymbol{\sigma}_n$, $\boldsymbol{\sigma}_c$ and $\boldsymbol{\sigma}_m$ the stress tensors for the cells, collagen and matrigel. Neglecting inertial effects, the momentum balance in each phase is given by:

$$\nabla \cdot (\phi_n \boldsymbol{\sigma}_n) + \boldsymbol{\kappa}_{cn} \cdot (\boldsymbol{v}_c - \boldsymbol{v}_n) - \kappa_{mn}(\boldsymbol{v}_n - \boldsymbol{v}_m) - \boldsymbol{F}_c - \boldsymbol{F}_m + p\nabla\phi_n = 0, \tag{3a}$$

$$\nabla \cdot (\phi_c \boldsymbol{\sigma}_c) - \boldsymbol{\kappa}_{cn} \cdot (\boldsymbol{v}_c - \boldsymbol{v}_n) - \boldsymbol{\kappa}_{cm} \cdot (\boldsymbol{v}_c - \boldsymbol{v}_m) + \boldsymbol{F}_c + p\nabla\phi_c = 0, \tag{3b}$$

$$\nabla \cdot (\phi_m \boldsymbol{\sigma}_m) + \boldsymbol{\kappa}_{cm} \cdot (\boldsymbol{v}_c - \boldsymbol{v}_m) + \kappa_{mn}(\boldsymbol{v}_n - \boldsymbol{v}_m) + \boldsymbol{F}_m + p\nabla\phi_n = 0, \tag{3c}$$

where $\boldsymbol{F}_c$ and $\boldsymbol{F}_m$ are the forces exerted on the collagen and matrigel respectively by the cells and $\boldsymbol{\kappa}_{cn}$, $\boldsymbol{\kappa}_{cm}$ and κ_{mn} specify the drag between phases. We allow $\boldsymbol{\kappa}_{cn}$ and $\boldsymbol{\kappa}_{cm}$ to be rank two tensors, as the drag between the collagen and other phases may be affected by the fibre orientation. We discuss the form of these drag terms below (see §2.2).

2.2 Constitutive relations

Cells are modelled as an incompressible inviscid fluid, so

$$\sigma_{n_{ij}} = -p_n \delta_{ij}, \tag{4}$$

where the pressure in the cells p_n is assumed to consist of a pressure p which is common to all the phases, together with an additional term, $f(\phi_n)$, which drives cell dispersion or aggregation. We therefore write

$$p_n = p + f(\phi_n). \tag{5}$$

A functional form for f must be proposed which describes the cell-cell behaviour appropriately. We anticipate cell-cell attraction (represented by a negative pressure) when the cell density is low and net cell-cell repulsion (represented as a positive pressure) at high cell densities.

We assume that the most important attribute of the collagen is its anisotropy, which is due to the presence of the fibrous microstructure. The simplest way of representing these properties would be to treat the collagen as a transversely isotropic material - *i.e.* a material having a single preferred direction (defined in this case by the fibre alignment at each point). Previous work suggests that collagen gels can be treated as compressible upper-convected Maxwell (UCM) fluids [15, 1, 21]. However, these studies neglected fibre orientation and treated the gel as isotropic. We note that values of the gel's shear modulus (G^*) and viscosity (μ^*) have been reported as $G^* = 1.185 \times 10^4$ dyne cm^{-2} and $\mu^* = 1.24 \times 10^8$ dyne s cm^{-2} [21]. Given a timescale for acini /

network formation, T^*, of the order of a few days ($10^5 - 10^6 s$) we find the Deborah number (ratio of the gel's relaxation timescale to T^*) to be $\mathcal{D} = \mu^*/G^*T^* \sim 0.1 - 0.01$. Hence on the timescale of interest, visous effects dominate. Combining this information, and ignoring compressibility effects for simplicity, we treat the collagen as an incompressible, transversely isotropic viscous fluid with the stress tensor [9, 10]

$$\sigma_{c_{ij}} = -p\delta_{ij} + 2\mu_c e^c_{ij} + \mu_1 a_i a_j + \mu_2 a_i a_j a_k a_l e^c_{kl} + 2\mu_3 \left(a_i a_l e^c_{jl} + a_j a_m e^c_{mi}\right), \tag{6}$$

where $\boldsymbol{a}(\mathbf{x}, t)$ is the fibre director field such that $|\boldsymbol{a}| = 1$, δ_{ij} is the Kronecker delta and $e^c_{ij} = \frac{1}{2}\left(\partial v_{ci}/\partial x_j + \partial v_{cj}/\partial x_i\right)$ is the rate-of-strain for the collagen. In equation (6), p is the fluid pressure, μ_c is the isotropic component of the viscosity (the matrix viscosity modified for the presence of the fibres; see [7] for more details), μ_1 gives the tension in the fibres, μ_2 is related to the extensional viscosity along the fibres and μ_3 gives the difference between the shear viscosity along the fibres and that transverse to them. Finally, we require an equation for the evolution of $\boldsymbol{a}$: as in [10], we assume fibres are advected, stretched and reoriented with the flow of the collagen, so

$$\frac{\partial \boldsymbol{a}}{\partial t} + \boldsymbol{v}_c \cdot \nabla \boldsymbol{a} + \left[\boldsymbol{a} \cdot (\boldsymbol{a} \cdot \nabla \boldsymbol{v}_c)\right] \boldsymbol{a} = \boldsymbol{a} \cdot \nabla \boldsymbol{v}_c. \tag{7}$$

We model the matrigel as an isotropic incompressible viscous fluid, with constant viscosity μ_m. Hence

$$\sigma_{m_{ij}} = -p\delta_{ij} + 2\mu_m e^m_{ij}, \tag{8}$$

where e^m_{ij} is the rate-of-strain for matrigel ($e^m_{ij} = \frac{1}{2}\left(\partial v_{mi}/\partial x_j + \partial v_{mj}/\partial x_i\right)$).

When prescribing the interphase drags we assume that there is no drag if either interacting species is absent, and that is depends on the fibre orientation when one of the species is collagen. We therefore propose

$$\kappa_{cn_{ij}} = \phi_c \phi_n \left(D_{cn}\delta_{ij} - d_{cn} a_i a_j\right), \tag{9a}$$

$$\kappa_{cm_{ij}} = \phi_c \phi_m \left(D_{cm}\delta_{ij} + d_{cm} a_i a_j\right), \tag{9b}$$

$$\kappa_{mn} = D_{mn}\phi_n \phi_m, \tag{9c}$$

such that the collagen-cell and collagen-medium drags consist of isotropic and anisotropic parts, whilst the medium-cell drag is only isotropic.

We now consider the force terms $\boldsymbol{F}_c$ and $\boldsymbol{F}_m$. We suppose that the force exerted on the collagen at a point $\boldsymbol{x}$ by the cells is the sum of the forces exerted by cells at surrounding points $\boldsymbol{x'}$, weighted by distance so that

$$\boldsymbol{F}_c(\boldsymbol{x}) = \int_\Omega G\left(\frac{\phi_c \boldsymbol{a}(\boldsymbol{x'}) \cdot (\boldsymbol{x} - \boldsymbol{x'})}{|\boldsymbol{x} - \boldsymbol{x'}|}\right)(\boldsymbol{x} - \boldsymbol{x'})\, F\left(\frac{|\boldsymbol{x} - \boldsymbol{x'}|}{\epsilon}\right)\phi_n(\boldsymbol{x'})\, d\boldsymbol{x'}. \tag{10}$$

In (10), F is a function which describes how the force exerted at point x depends upon the distance between that point and the position of the cell, x'. G describes the degree to which forces are transmitted more effectively along fibres than through non-fibrous matrix material: when the direction of the force $(x - x')$ is aligned with the fibres, its magnitude is maximised. When the range of influence of the cells on the collagen is small ($\epsilon \ll 1$), the integrand in equation (10) can be expanded as a Taylor series and integrated term by term. This gives a force of the form

$$\boldsymbol{F}_c(\boldsymbol{x}) = \beta \nabla \phi_n + \gamma \phi_c^2 \boldsymbol{a}\left[(\boldsymbol{a} \cdot \nabla)\, \boldsymbol{\phi_n}\right], \tag{11}$$

where β, γ are constants which depends on G and the geometry of the region Ω. We assume that the forces are mainly transmitted through the fibres and hence there are no constant terms within G; thus we take $\beta = 0$. For simplicity we assume that the force exerted by the cells on the matrigel can be neglected in which case

$$\boldsymbol{F}_m = 0. \tag{12}$$

2.3 Initial and boundary conditions

We prescribe the initial distributions of cells, collagen and medium, $\phi_n(\mathbf{x}, t = 0) = \phi_{n0}(\mathbf{x})$, $\phi_c(\mathbf{x}, t = 0) = \phi_{c0}(\mathbf{x})$ and $\phi_m(\mathbf{x}, t = 0) = \phi_{m0}(\mathbf{x})$, (subject to the constraint (1)) as well as an initial condition for $\boldsymbol{a}$, $\boldsymbol{a}(\mathbf{x}, t = 0) = \boldsymbol{a}_0(\mathbf{x})$. We assume that the cells, collagen and medium are constrained to remain within the culture well. Hence, on the boundaries on the well, which we denote to lie at $\boldsymbol{x} = \boldsymbol{\Gamma}$, we set

$$\boldsymbol{v}_n(\boldsymbol{\Gamma}, t) = \boldsymbol{v}_c(\boldsymbol{\Gamma}, t) = \boldsymbol{v}_m(\boldsymbol{\Gamma}, t) = 0. \tag{13}$$

We therefore have ten equations (2-8) in the ten unknowns ϕ_n, ϕ_m, ϕ_c, $\boldsymbol{v}_n$, $\boldsymbol{v}_m$, $\boldsymbol{v}_c$, $\boldsymbol{\sigma}_n$, $\boldsymbol{\sigma}_m$, $\boldsymbol{\sigma}_c$, $\boldsymbol{a}$, subject to the constraint (1).

3 Linearised analysis for sparsely-seeded cells

Our model consists of a system of coupled nonlinear PDEs, and it is thus difficult in general to make any progress investigating them analytically. The difficulty is further compounded because we cannot simplify to a one-dimensional geometry: this would eliminate the effects of anisotropy, which plays an important role in the observed behaviour. In this section, we make a number of other simplifications, which allow us to reduce the complexity of the model. In particular we assume that the cells are seeded sparsely in the matrix, and so (at least for short times) the cell volume fraction will be small. We introduce a small parameter, $\epsilon \ll 1$, which represents a typical value of the (small) cell volume fraction. In addition we ignore the anisotropic components of the drag coefficients $\boldsymbol{\kappa}_{cn}$ and $\boldsymbol{\kappa}_{cm}$ - so that $d_{cn} = d_{cm} = 0$ in equations (9). We expand all dependent variables as regular power series in ϵ so that $\phi_c = \phi_c^{(0)} + \epsilon\phi_c^{(1)} + ...$ It is straightforward to show that the leading-order solution is then

$$\phi_n^{(0)} = 0, \qquad \phi_c^{(0)} = \phi_{c0}\left(\boldsymbol{x}\right), \qquad \phi_m^{(0)} = \phi_{m0}\left(\boldsymbol{x}\right) = 1 - \phi_{c0}\left(\boldsymbol{x}\right), \tag{14}$$

$$\mathbf{v}_m^{(0)} = 0, \qquad \mathbf{v}_c^{(0)} = 0, \qquad p^{(0)} = 0, \tag{15}$$

$$\mathbf{a}^{(0)} = \mathbf{a}_0\left(\boldsymbol{x}\right), \qquad \boldsymbol{\sigma}_c^{(0)} = 0, \qquad \boldsymbol{\sigma}_m^{(0)} = 0. \tag{16}$$

We show below how the leading-order cell velocity, $\boldsymbol{v}_n^{(0)}$, is determined by considering the equation for the correction to the cell density, $\phi_n^{(1)}$. We assume that the effects of cell dispersion/aggregation appear at this level, so that $f(\phi_n^{(1)}) = O(1)$ in equation (5).

At $O(\epsilon)$ we obtain equations for $\phi_n^{(1)}$ and $\boldsymbol{v}_n^{(0)}$ from the mass and momentum equations for the cells

$$\frac{\partial \phi_n^{(1)}}{\partial t} + \nabla \cdot \left(\phi_n^{(1)} \mathbf{v}_n^{(0)}\right) = S\phi_n^{(1)}\phi_c^{(0)}, \tag{17}$$

$$\phi_n^{(1)}\mathbf{v}_n^{(0)} = -\frac{1}{k_{cn}\phi_c^{(0)} + k_{mn}\phi_m^{(0)}} \left(\left(f(\phi_n^{(1)}) + \phi_n^{(1)} f'(\phi_n^{(1)})\right) \nabla\phi_n^{(1)} + \gamma {\phi_c^{(0)}}^2 \mathbf{a}^{(0)} \left(\mathbf{a}^{(0)} \cdot \nabla\phi_n^{(1)}\right)\right), \tag{18}$$

where $f'(\phi_n^{(1)}) = df(\phi_n^{(1)})/d\phi_n^{(1)}$. Combining (17, 18) gives

$$\frac{\partial \phi_n^{(1)}}{\partial t} = \nabla \cdot \left(\boldsymbol{\mathcal{D}} \cdot \nabla\phi_n^{(1)}\right) + S\phi_n^{(1)}\phi_c^{(0)}, \tag{19a}$$

where the cell dispersion tensor $\boldsymbol{\mathcal{D}}$ has components

$$\mathcal{D}_{ij} = \frac{1}{k_{cn}\phi_c^{(0)} + k_{mn}\phi_m^{(0)}} \left\{\left[f\left(\phi_n^{(1)}\right) + \phi_n^{(1)} f'\left(\phi_n^{(1)}\right)\right]\delta_{ij} + \gamma {\phi_c^{(0)}}^2 {a_i}^{(0)} {a_j}^{(0)}\right\}. \tag{19b}$$

Hence, in the case of sparsely seeded cells, our model reduces to a diffusion equation for cell movement. However, a novel feature of equation (19a), compared to existing mathematical models, is that the diffusion is both anisotropic (depending upon the fibre orientations) and nonlinear (depending upon the cell volume fraction). The evolution of $\phi_n = \epsilon\phi_n^{(0)}$ depends on $\mathbf{a}^{(0)}$ only, *i.e.* on the initial fibre configuration. We need to go to higher-order terms to determine the influence of the feedback of the cells on fibre orientation.

4 Conclusions and future work

During the study group, we formulated a new model for pattern formation in cells grown in collagen and matrigel *in vitro*. The inclusion of the effects of anisotropy, arising from the presence of the fibre in the collagen gel, is a novel aspect of the modelling. Owing to the fact that the model consists of system of nonlinear partial differential equations, it was difficult to make much progress in the analysis or simulation of the model within the time constraints of the study group. When the cell seeding is non-sparse (*i.e.* order one), then the full balance is required. However, a linearised analysis for the case of sparsely seeded cells was able to provide some insights; the fibre orientation influences the cell distribution with more rapid cell motility in the fibre direction, but higher-order terms are needed to determine the feedback of cells on the fibre orientation. We note from equation (19) that the movement of the cells is driven by the $f(\phi_n)$ term, which is the part of the the cell

pressure, p_n, describing the effect of cell-generated forces on the cells themselves: this is typical of multiphase models like ours [3]. However, in this model, cell diffusion is enhanced along the fibre direction, as represented by the γ term in (19). This enhanced diffusive effect is connected with the strength of the forces exerted by the cells on the fibres. Obviously, further analysis and refinement of the model presented here is required, but we believe it provides a useful starting point for future work.

Before any quantitative predictive models of cell-matrix interactions can be developed, a great deal of further experimental and theoretical work will need to be undertaken. In particular, the mechanics of anisotropic materials such as collagen will need to be much better understood. For the simple transversely isotropic viscous fluid model used here, we need to know four parameters (the μ's). However, at the moment an experimental protocol for measuring these parameters has not been developed. Recent work on extensional and squeezing flows [10, 6] suggests ways in which some of these values may be determined, but further theoretical work is still needed. However, the viscous model is itself a simplification of the real material properties, since viscoelastic effects, the effects of fibre-fibre interactions, fibre entanglement, cross-linking *etc.* probably need to be taken in account in practice. Similarly, more detailed measurements of the forces exerted by cells on the matrix, and how they are transmitted along the fibres are needed: at present the forms assumed for these terms are simply based on surmise. The following phenomenological form for $\boldsymbol{F}_c$ was also suggested

$$\boldsymbol{F}_c = \theta\phi_c \left[(\boldsymbol{a}\cdot\nabla)\, \phi_n \boldsymbol{a} \right],$$

where θ is constant. This alternative form has the advantage that, even when there is no variation in the fibre alignment $\boldsymbol{a}$, a spatial variation in the cell density will generate a force. We postpone consideration of this case for future work.

Other extensions to the basic model presented here would be to include the effects of diffusible chemical (such as growth factors, chemoattractants, *etc.*) on cell behaviour, or to extend the model of gel mechanics in other ways, *e.g.* by allowing for a distribution of fibre directions, instead of the single direction assumed here; the effects of fibroblasts could then be incorporated. A simple way of doing this would be to introduce an order parameter together with an empirical equation for its evolution, as in [17]. Alternatively, the evolution of the microstructure can be considered in greater detail by introducing a density function which gives the probability of finding a fibre aligned along a unit vector $\boldsymbol{a}$ at a point $\boldsymbol{x}$ in the material at time t - see *e.g.* [11, 20]. However, the latter especially, would make the model much more complicated. Another potential research direction would be to explore the use of agent-based models for the cells, coupled with the continuum mechanical models for the collagen and matrigel. This type of model could provide a more biologically realistic description of cell behaviour, as it would allow cells to be represented as discrete objects; subcellular mechanisms would then be included. In addition, the low cell-seeding density indicates such a model could be more applicable than the multiphase model which treats the cells as a continuum.

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