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A new concept for multiscale modelling of growing cell cultures

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Abstract

Dynamics of growing or actively deforming tissues in biological systems cannot be understood without taking mechanical interactions into account. In this work, we propose a new multiscale approach for modelling growing tissues using analytical up-scaling techniques originally developed for crystals. Adopting this approach corresponding macroscopic continuum models can be derived on the basis of the microscopic models (individual based models). Assuming isotropy these macroscopic models based on energy functionals can be formulated in the framework of multiple natural configurations often used in modelling growing tissues. In the case of anisotropic growth our ansatz shows that constitutive relations depending only on mechanical deformations, as in the case of isotropic growth, are not sufficient. They depend also on the growth itself. The explicit form of the dependence can be recovered via homogenisation formulae inheriting most details of the microscopic models. This new concept of a multiscale modelling approach unifying individual based and sub-cellular element models with continuum models offers an new perspective for mathematical modelling and simulation in many biological systems.

 $Keywords:\ {\tt Growth},\ {\tt Multiscale\ modelling},\ {\tt Homogenisation},\ {\tt Multiple\ natural\ configurations}.$

AMS Subject Classification: 35B27, 35Q74, 35Q92, 74Q05, 74Q15

1. Introduction.

The generation and organisation of multi-cellular tissues is one of the most fundamental questions in biology. Properties of biological tissues are determined by complex interactions between biomechanical and biochemical processes on multiple temporal and spatial scales: ranging from $\sim 10^{-9}$ s and $\sim 10^{-9}$ m for molecular processes to $\sim 10^7$ s and $\sim 1m$ for the development of organisms [1]. Due to their complexity developmental mechanisms

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leading to the generation of form in multi-cellular tissues can hardly be understood by *in vivo* or *in vitro* experiments alone. Mathematical modelling is an extremely useful tool to deal with this curse of complexity.

Depending on the temporal and spatial scales of interest different approaches to multi-cellular tissues can be found in the literature (cf.Fig. 1): These range from sub-cellular element models (e.g. [1,2]; modelling sub-cellular elements interacting through phenomenological potentials) and single-cell-based models (e.g. [3–6]; modelling cells as single deformable polygons or ellipsoids) to continuum models (e.g. [7–15]; considering local densities of cells). On the one hand, processes on sub-cellular or cellular scales play a crucial role in determining the observed phenomena leading to a preference of microscopic models based on first principles. On the other hand, one is interested in phenomena typically involving hundreds or thousands of cells, indicating a preference for continuum models requiring less computational effort. Since macroscopic models are typically of a heuristic type, one of the central challenges in mathematical approaches is how macroscopic models might be linked with microscopic models or even rigorously derived from microscopic models.



Fig. 1. Length scales and corresponding models with their degree of freedom in morphogenesis (courtesy to T. Newman, Arizona State University)

Based on a cellular automaton approach, [16] has derived a macroscopic description of growing cell cultures in the framework of reactiondiffusion systems by computational means. Comparing computations of

reaction-diffusion equations and off-lattice cellular automata models functional dependencies of the continuous reaction-diffusion systems have been determined. The approach indicates how macroscopic approaches to tissues can be guided by microscopic models. However, the approach makes quite strong a priori assumptions on the macroscopic continuum model: a priori it is assumed that it is of a reaction-diffusion type. Rigorous derivations of macroscopic models based on microscopic models would offer the chance to obtain appropriate continuum models avoiding strong a priori assumptions. Recently [17–19] have proposed rigorous multiscale frameworks for elastic materials. Based on discrete atomistic interactions constitutive relations are derived. Using homogenisation formulae macroscopic properties can be directly linked to microscopic properties. Extending this approach for "passive" materials to biological "active" materials, e.g. growing tissues, seems to be highly promising with respect to modelling mechanobiological phenomena.

In this work, we show how the rigorous approach of [17] could be extended to active biological materials. That is, we show how to derive continuum macroscopic active material laws on the basis of a well studied microscopic description. To do so, we restrict us to a relative simple 2D microscopic model for epithelial mono layers considering single cells as deformable polygons. The model, given in terms of energy functionals, is related to a large number of discrete models [1-6]. For this model, we then derive an appropriate macroscopic model under the assumption of a time scale separation between "passive" and "active" behaviour. The derivation is based on the results of [17] using the concept of Γ -convergence. Like the microscopic model also the macroscopic model is based on a description via energy functionals. Using variational calculus corresponding elastic continuum mechanical models can be derived. Considering isotropic growth, the results agree perfectly with the so called notion of multiple natural configurations [7,20]. In the case of anisotropic growth the results indicate that the consideration of remodelling, i.e. the evolution of material properties, is important. The constitutive relations have to depend additionally on the growth tensor. A fact commonly ignored in most approaches based on the notion of multiple natural configurations.

2. A discrete cell based model.

Discrete microscopic models are a popular theoretical approach to growing tissues. Cells are typically modelled as deformable quasi-spherical parti-

cles (e.g. [3]), deformable ellipsoids (e.g. [6]), or polygons (e.g. [5]). Mechanical interactions are encoded using energy functionals. Thus, neglecting any dynamics, the stationary shape or deformation of a cell culture at any time is given by the corresponding energy minimum. In the following, we consider a simplified 2D model. Most other discrete models (e.g. [1–6]) could be formulated in a similar way. An extension of the approach to 3D is straight forward.

2.1. The discrete model

Model 2.1. Let us consider a discrete cell culture in Ω , as shown in Fig. 2, where ε is the typical length scale of a cell. For any given time t the quasi-stationary shape / deformation of the culture minimises the discrete free energy

$$E^{\varepsilon}(t) = \sum_{i \in cells} V_i(t) \Big(E_{perimeter}(i;t) + \sum_{j \in links} E_{link}(i,j;t) \Big),$$

where $V_i(t)$ is the volume of cell *i* in the undeformed state. The energies $E_{perimeter}(t)$ and $E_{link}(t)$ model cytoskeletal tension on the surface and within the cell. The energies depend on the position of the cell centres and vertices of the polygonal cells.

The scaling with the undeformed volume $V_i(t)$ of the *i*th cell at time *t* is the natural scaling: the energy is stored in the whole cell. If a cell is growing, we do not expect the energy density of the cell to decrease. For simplicity we assume that the energies $E_{\text{perimeter}}$ and E_{link} are of the following quadratic form:

(1)
$$E_{\text{perimeter}}(\mathcal{P}_i(t), P_i(t)) = \kappa_P \Big(\frac{\mathcal{P}_i(t)}{P_i(t)} - 1\Big)^2,$$

(2)
$$E_{\text{link}}(\mathcal{L}_{i,j}(t), L_{i,j}(t)) = \kappa_L \left(\frac{\mathcal{L}_{i,j}(t)}{L_{i,j}(t)} - 1\right)^2,$$

depending on cell perimeters $\mathcal{P}_i(t)$ and link lengths $\mathcal{L}_{i,j}(t)$ in the deformed configuration as well as on the same quantities in the relaxed / undeformed state $P_i(t)$, $L_{i,j}(t)$. κ_P and κ_L are the corresponding mechanical moduli. Growth is included in Model 2.1 via the time dependence of the parameters $P_i(t)$ and $L_{i,j}(t)$, and $V_i(t)$ (cf. Section 2.2). Model 2.1 can also be extended to include volume compressibility of the cytosol explicitly. However, from a mathematical point of view the contributions of volume compressibility is rather less interesting since it is a purely isotropic contribution.



Fig. 2. Illustration of the geometric setup used in the microscopic discrete Model 2.1. The initial configuration of a culture is shown, where ε is the order of the cell size. Roman indices denote cell centres and Greek indices denote the vertices of the polygonal cells.

The basic variables describing cell perimeters $\mathcal{P}_i(t)$ and link lengths $\mathcal{L}_{i,j}(t)$ can be expressed in terms of the position of the cell centres \boldsymbol{x}_i , the vertices of the underlying network, after deformation for all times t:

$$\boldsymbol{x}(t) \equiv \boldsymbol{\chi}^{arepsilon}(t) : \mathbb{R}^2 \ni \boldsymbol{X} \mapsto \boldsymbol{\chi}^{arepsilon}(\boldsymbol{X};t) \in \mathbb{R}^2,$$

which are the "natural" degrees of freedom of Model 2.1. (Since we are interested in mechanics, we will restrict ourselves to deformations preserving the orientation.) The superscript ε indicates that we are working on a discrete lattice. That is, we are only interested in the values of χ^{ε} at discrete points.

Let us make the relation between $\mathcal{P}_i(t)$ and the deformation χ more precise (cf. also Fig. 2). In the following Roman indices will denote cell centres and Greek indices will denote vertices of the polygonal cells, i.e. the points where three cells touch each other. For each cell *i* this set of points will be denoted $\mathcal{T}_i^{\varepsilon}$. Considering the initial configuration, $\boldsymbol{\xi}_{i,j}$ denotes the vector (of length $L_{i,j}^0$) connecting two cell centres. It is the natural vector of the underlying network. $\boldsymbol{\zeta}_{i,\alpha}$ (of length $L_{i,\alpha}^0$) is the vector connecting the cell centre with one of the points in $\mathcal{T}_i^{\varepsilon}$. (The vector $\boldsymbol{\zeta}_{i,\alpha}$ can be decomposed by a combination of the vectors $\boldsymbol{\zeta}_{i,j}$ and $\boldsymbol{\zeta}_{i,k}$ with *i* and *k* appropriately, i.e. $\boldsymbol{\zeta}_{i,\alpha} = (\boldsymbol{\xi}_{i,j} + \boldsymbol{\xi}_{i,k})/3)$. $\boldsymbol{\zeta}_{\alpha,\beta}$ is the vector (of length $L_{\alpha,\beta}^0$) connecting two neighbouring points in $\mathcal{T}_i^{\varepsilon}$. (The vector $\boldsymbol{\zeta}_{\alpha,\beta}$ can be decomposed by a combination of the vectors $\boldsymbol{\zeta}_{i,\beta}$, i.e. $\boldsymbol{\zeta}_{\alpha,\beta} = -\boldsymbol{\zeta}_{i,\alpha} + \boldsymbol{\zeta}_{i,\beta}$). Furthermore, $V_{i,\beta,\gamma}$ denotes the triangular area spanned by *i*, β and γ in the undeformed configuration.

Considering the deformed configuration, it holds (caligraphic quantities are measured after the deformation)

(3)
$$\mathcal{L}_{i,j}(t) \equiv \mathcal{L}_{i,j}(\boldsymbol{\chi}(t)) = \frac{|\boldsymbol{\chi}(\boldsymbol{X}_i + \boldsymbol{\xi}_{i,j}; t) - \boldsymbol{\chi}(\boldsymbol{X}_i; t)|}{|\boldsymbol{\xi}_{i,j}|} L^0_{i,j}$$
$$= |D^{\boldsymbol{\xi}_{i,j}} \boldsymbol{\chi}(\boldsymbol{X}_i; t)| L^0_{i,j},$$

 $\mathcal{L}_{i,\alpha}(\boldsymbol{\chi}(t))$ and $\mathcal{L}_{\alpha,\beta}(\boldsymbol{\chi}(t))$ similar. Here $D^{\boldsymbol{\xi}}\boldsymbol{\chi}(\boldsymbol{X}) \equiv (\boldsymbol{\chi}(\boldsymbol{X} + \boldsymbol{\xi}) - \boldsymbol{\chi}(\boldsymbol{X}))/|\boldsymbol{\xi}|$ is the discrete finite difference quotients in direction of the vector $\boldsymbol{\xi}$. Using these relations $\mathcal{P}_i(t)$ can be formulated similarly in terms of vertex deformations:

$$\mathcal{P}_i(t) \equiv \mathcal{P}_i(\boldsymbol{\chi}(t)) = \sum_{\alpha \in \mathcal{T}_i^{\varepsilon}} \mathcal{L}_{\alpha, \alpha+1}(\boldsymbol{\chi}(t)).$$

The perimeter P_i in the undeformed configuration is defined analogously. For V_i it holds $V_i(t) = \sum_{\alpha \in \mathcal{T}_i^{\in}} V_{i,\alpha,\alpha+1}(t)$. Thus Model 2.1 is formulated in terms of lengths $\mathcal{L}_{i,j}$ and $\mathcal{L}_{\alpha,\alpha+1}$, which itself can be formulated in terms of the deformation $\boldsymbol{\chi}$.

Although Model 2.1 holds for any kind of geometry, we will restrict us in the following for mathematical reasons (cf. Section 3) to a purely hexagonal culture as shown in Fig. 2. In general, this is obviously not true, however many tissues exhibit patterns close to a perfect hexagonal structure [21]. Cells are packed in the most efficient way.

Equation (3) as well as $\mathcal{L}_{i,j}$, \mathcal{P}_i and \mathcal{V}_i depend on finite differences D^{ξ} of the deformation of vertices of the underlying network. In a mechanical framework the use of deformation gradients \mathbf{F}^{ε} (commonly used in continuum mechanics) is rather natural and thus preferable. It holds

(4)
$$D^{\boldsymbol{\xi}}\boldsymbol{\chi}(\boldsymbol{X};t) = \mathbf{F}^{\varepsilon} \cdot \frac{\boldsymbol{\xi}}{|\boldsymbol{\xi}|}$$

with the deformation tensor

$$\mathbf{F}^{\varepsilon}(\boldsymbol{X};t) = \nabla_{\boldsymbol{X}}\boldsymbol{\chi}.$$

Here, ∇_X is the gradient with respect to the initial configuration, i.e. the Lagrangian coordinate system. Again, the superscript ε indicates that we work within a discrete setup.

2.2. Biological growth

In many biologically relevant cases growth is spatially varying and sometimes even anisotropic. Typically, growth takes place on time scales of several minutes, whereas mechanical relaxation / mechanical interactions take

place on much faster time scales. Thus, one typically assumes that with respect to mechanics biological tissues are in a quasi steady state on time scales relevant for growth. That is, we can introduce growth in Model 2.1 by assuming that $V_i(t)$, $P_i(t)$ as well as $L_{i,j}(t)$ (respectively the corresponding vectors $\boldsymbol{\xi}_{i,j}$) are time dependent variables, whose evolution is directly determined by growth.

In microscopic discrete models, cells grow and once they are sufficiently large cell division is initiated. Cell division will be omitted in our approach, i.e. the topology is constant and does not change due to growth. Omitting cell division is a major assumption. However, starting with a perfectly hexagonal cell culture, cell division would imply a non-perfect symmetry. This in turn, would anticipate the mathematical techniques introduced in Section 3.

2.3. The discrete notion of multiple natural configurations

The notion of multiple natural configurations is a concept introduced for continuum mechanical systems with evolving rest configurations. It has been originally introduced for problems in thermo-elasticity and elastoplasticity (for a review see e.g. [20]). As a description of biological growth it has been first applied by [7] and since then used in many theoretical approaches to growing tissues, e.g. [8–15].



Fig. 3. The multiplicative decomposition of the deformation gradient $\mathbf{F}^{\varepsilon} = \mathbf{F}^{\varepsilon, \text{mech}} \cdot \mathbf{G}$

The main idea is to decompose the deformation tensor $\mathbf{F}^{\varepsilon} \in \mathbb{R}^{2 \times 2}$ into

two consecutive deformations (cf. Fig. 3):

$$\mathbf{F}^{\varepsilon} = \mathbf{F}^{\varepsilon, \text{mech}} \cdot \mathbf{G}.$$

 $\mathbf{F}^{\mathrm{mech}} \in \mathbb{R}^{2 \times 2}$ is the deformation tensor due to mechanics. The tensor $\mathbf{G}(\mathbf{X};t) \in \mathbb{R}^{2 \times 2}$, which is assumed to be invertible, is a map from a reference state, e.g. the initial force-free configuration, to the current natural force-free configuration. The tensor \mathbf{G} can be directly related to growth, i.e. we assume that growth can be modelled locally as a linear map. Growth is completely determined by specifying the two main axes of growth and the corresponding growth rates, i.e. the two eigenvectors and corresponding eigenvalues of \mathbf{G} . The specific form and evolution of the tensor \mathbf{G} has to be modelled. The growth rate, e.g. obtained by a complex network of biochemical control pathways, determines only one of the invariants of the growth tensor, namely $J^G = \det \mathbf{G}$. All other components have to be predicted using available biological knowledge.

As outlined in Section 2.1, the key ingredients of the model are the lengths $\mathcal{L}_{i,j}(t)$ and $\mathcal{L}_{\alpha,\alpha+1}(t)$ as well as $L_{i,j}(t)$, $L_{\alpha,\alpha+1}(t)$, and $V_{i,\alpha,\alpha+1}(t)$, respectively the corresponding vectors $\boldsymbol{\xi}_{i,j}$. Let us assume that the vector $\boldsymbol{\xi}_{i,j}$ in the initial force-free configuration is affected by growth in the following way, i.e. it has the following form in the grown configuration:

(5)
$$\hat{\boldsymbol{\xi}}_{i,j}(t) = \mathbf{G}(\boldsymbol{X}; t) \cdot \boldsymbol{\xi}_{i,j}$$

and $\hat{\boldsymbol{\zeta}}_{i,\alpha}(t), \, \hat{\boldsymbol{\zeta}}_{\alpha,\beta}(t)$ similar. Hence, we find

(6)
$$L_{i,j}(t) = |\mathbf{G}(\boldsymbol{X};t) \cdot \boldsymbol{\xi}_{i,j}|,$$

(7)
$$V_{i,\alpha,\alpha+1}(t) = J^G(\boldsymbol{X};t)V_{i,\alpha,\alpha+1}(0) \equiv \det\left(\mathbf{G}(\boldsymbol{X};t)\right)V_{i,\alpha,\alpha+1}(0),$$

and $L_{\alpha,\alpha+1}(t)$ similar. The approach implies that growth of the single components is not completely independent, it is only independent along orthogonal directions. This is a realistic biological assumption. Otherwise growth of a single cell outside any culture would imply the generation of pre-stress [22], which is typically not a phenomenon due to growth but rather an intrinsic property of cells and should be conserved under growth.

Energies (1)-(2) depend on relative deformations, thus growth solely enters the model by affecting the relative deformations. Using the notion of the growth tensor (5)-(7) we find for the direct links

(8)
$$\frac{\mathcal{L}_{i,j}(t)^2}{L_{i,j}(t)^2} = \frac{(\mathbf{F}^{\varepsilon}(t) \cdot \boldsymbol{\xi}_{i,j})^T \cdot (\mathbf{F}^{\varepsilon}(t) \cdot \boldsymbol{\xi}_{i,j})}{|(\mathbf{G}(t) \cdot \boldsymbol{\xi}_{i,j})|^2},$$

 $\mathcal{L}_{\alpha,\alpha+1}$ and $L_{\alpha,\alpha+1}$ analogous, as well as $\mathcal{P}_i(t)/P_i(t)$ (which is simply composed of lengths). To compare the framework of multiple natural configurations in a discrete and continuous setting let us assume for simplicity that growth is overall constant, i.e. **G** is independent of **X**. The construction of **G** ensures that **G** is invertible, thus we find for (8) dropping t for convenience:

$$\frac{\mathcal{P}_i}{P_i} = \frac{\sum_{\alpha \in \mathcal{T}^{\varepsilon}_i} \left[(\mathbf{G} \cdot \boldsymbol{\xi}_{\alpha, \alpha+1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1}) \cdot (\mathbf{G} \cdot \boldsymbol{\xi}_{\alpha, \alpha+1}) \right]^{1/2}}{\sum_{\alpha \in \mathcal{T}_i^{\varepsilon}} |\mathbf{G} \cdot \boldsymbol{\xi}_{\alpha, \alpha+1}|},$$
$$\frac{\mathcal{L}_{i,j}^2}{L_{i,j}^2} = \frac{(\mathbf{G} \cdot \boldsymbol{\xi}_{i,j})^T}{|\mathbf{G} \cdot \boldsymbol{\xi}_{i,j}|} \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1}) \cdot \frac{(\mathbf{G} \cdot \boldsymbol{\xi}_{i,j})}{|\mathbf{G} \cdot \boldsymbol{\xi}_{i,j}|}.$$

2.3.1. Isotropic growth

In the case of isotropic growth, i.e. $\mathbf{G} = \gamma \mathbf{I}$, the relations above can be simplified significantly:

$$\frac{\mathcal{P}_i}{P_i} = \frac{\sum_{\alpha \in \mathcal{T}_i^{\varepsilon}} (\boldsymbol{\xi}_{\alpha,\alpha+1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1}) \cdot (\boldsymbol{\xi}_{\alpha,\alpha+1})}{P_i(0)}$$
$$\frac{\mathcal{L}_{i,j}^2}{L_{i,j}^2} = \frac{\boldsymbol{\xi}_{i,j}^T}{|\boldsymbol{\xi}_{i,j}|} \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1})^T \cdot (\mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1}) \cdot \frac{\boldsymbol{\xi}_{i,j}}{|\boldsymbol{\xi}_{i,j}|}.$$

Thus, mechanical energy densities per mass/volume $E^{\varepsilon}(t)/V(t) = E^{\varepsilon}(t)/(V(0) \cdot J^G(t))$ with $J^G(t) = \det \mathbf{G} = V(t)/V(0)$ in Model 2.1 depend solely on the mechanical deformation tensor $\mathbf{F}^{\varepsilon, \text{mech}} = \mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1} = \frac{1}{\gamma} \mathbf{F}^{\varepsilon}$ and not on the "true" deformation tensor \mathbf{F}^{ε} . In the case of isotropic growth, we find a perfect agreement with the so-called notion of multiple natural configurations [20].

2.3.2. Anisotropic growth

Considering anisotropic growth Model 2.1 differs significantly from typical approaches in the framework of multiple natural configurations [20]: Rewriting relation (8) in terms of mechanical deformations $\mathbf{F}^{\varepsilon,\text{mech}} = \mathbf{F}^{\varepsilon} \cdot \mathbf{G}^{-1}$, we obtain a formulation for the energy density $E^{\varepsilon}(t)/V(t)$ which depends on mechanical deformations $\mathbf{F}^{\varepsilon,\text{mech}}$ as well as the growth tensor \mathbf{G} . A further simplification is not possible. However, in current approaches using the concept of multiple natural configurations, the energy typically depends solely on $\mathbf{F}^{\varepsilon,\text{mech}}$, i.e. growth does not modify the postulated constitutive relations.

Thus, already our simple setup shows that in the case of anisotropic growth it is necessary to consider not only the "right" deformations, i.e. $E^{\varepsilon}(\mathbf{F}^{\varepsilon,\text{mech}})$, but also an evolution of the constitutive relations, i.e. $E^{\varepsilon}(\mathbf{F}^{\varepsilon,\text{mech}},\mathbf{G})$. This can be interpreted as a remodelling of the biological material induced by growth. The latter is often neglected and constitutive relations are assumed to be independent of growth.

3. From discrete to continuum

Above, we have introduced a simple microscopic discrete model for quasi-2D epithelial cell cultures. Considering large cell cultures a continuum description is preferable in many applications. That is, cell cultures are modelled as a continuous material. Here we propose a new modelling framework based on the results of [17].

To do so, let us summarise the discrete model outlined so far: we consider a cell culture with tightly adhering cells exhibiting a symmetric hexagonal pattern. Growth, which we assume without loss of generality to be (locally) constant, is only included via growth of cells / sub-cellular elements sizes; cell fission is neglected. Furthermore, we assume that mechanics are in a quasi-steady state with respect to growth. The mechanical behaviour of the culture is modelled by the discrete energy functional

(9)
$$E^{\varepsilon}(t) = \sum_{i \in \text{cells} \subset \Omega} J_i^G(t) \left(E_{\text{perimeter}} \left(\sum_{\alpha \in \mathcal{T}_i^{\varepsilon}} |\mathbf{F}^{\varepsilon}(t) \cdot \boldsymbol{\zeta}_{\alpha, \alpha+1}|, P_i(t) \right) + \sum_{j \in \text{links}} E_{\text{link}} \left(|\mathbf{F}^{\varepsilon}(t) \cdot \boldsymbol{\xi}_{i,j}|, L_{i,j}(t) \right) \right),$$

with $J_i^G(t) = V_i(t)/V_0(t)$. The actual shape of the culture is given by the deformation $\chi_{\min}^{\varepsilon}$ minimising $E^{\varepsilon}(t)$. To facilitate further analysis, let us assume $\chi^{\varepsilon} \in \mathcal{F}^{\varepsilon}$ and thus also $\chi_{\min}^{\varepsilon} \in \mathcal{F}^{\varepsilon}$ with

 $\mathcal{F}^{\varepsilon}(\Omega) \equiv \{ \boldsymbol{\chi}^{\varepsilon} : \Omega \to \mathbb{R}^2 : \boldsymbol{\chi}^{\varepsilon} \text{ is linear on} \\ \text{ each cell of the underlying discrete lattice } \}.$

That is the discrete space, i.e. the finite dimensional $\chi^{\varepsilon} : \mathcal{G}^{\varepsilon} \cap \Omega \to \mathbb{R}^2$ maps, is embedded into an infinite dimensional continuous space, i.e. maps $\chi^{\varepsilon} : \Omega \to \mathbb{R}^2$ piecewise linear on each triangular cell of the lattice $\mathcal{G}^{\varepsilon}$.

Neglecting contributions due surface compressibility, i.e. $\kappa_P = 0$, the model reduces to simple pair interactions. Under this assumption E^{ε} is a

special case of free energies describing atomistic interactions in crystal lattices. The continuum limits of such energies have been studied in several works using Γ -convergence [17] as well as other techniques [18,19]. Here we will rely on the approach of Alicandro and Cicalese [17] using Γ -convergence results from the theory of homogenisation of integrals [23]. This abstract approach allows to prove the existence of appropriate continuum limit energy functionals. Furthermore, they show that considering periodic microscopic geometries and assuming convex energies the continuum functionals can be characterised directly via homogenisation formulae.

3.1. Existence of a continuum limit

Following the work of Alicandro and Cicalese [17] the following conjecture should hold true:

Conjecture 3.1. For all times t and for every sequence (ε_j) of positive real numbers converging to 0, there exists a sub-sequence (ε_{j_k}) and a continuous quasi-convex function $\Psi : \mathbb{R}^{2\times 2} \to [0,\infty)$, such that $(E^{\varepsilon_{j_k}}(t))$, specified in equation (9), Γ -converges with respect to the $L^2(\Omega; \mathbb{R}^2)$ -topology to E(t) : $L^2(\Omega; \mathbb{R}^2) \to [0,\infty]$ defined as

$$E(\boldsymbol{\chi}(t),t) = \begin{cases} \int_{\Omega} J^{G}(t) \Psi(\nabla_{X} \boldsymbol{\chi}(t),t) d\boldsymbol{X} & \text{if } \boldsymbol{\chi}(t) \in W^{1,2}(\Omega;\mathbb{R}^{2}) \\ \infty & \text{otherwise,} \end{cases}$$

with $J^G(t)$ being the relative local volume change due to growth defined as above.

Here, $W^{1,2}(\Omega; \mathbb{R}^2)$ is the standard Sobolev space [24]. The scaling with $J^G(t)$ ensures that in the case of isotropic growth our approach agrees with the notion of multiple natural configurations (cf. Section 2.3 and Section 4.1), since $\int_{\Omega} \cdot J^G(t) d\mathbf{X}$ is an integral over the grown configuration.

The embedding of $W^{1,2}(\Omega; \mathbb{R}^2)$ to $L^2(\Omega; \mathbb{R}^2)$ is compact using the standard definition of L^2 spaces [24]. Hence, Γ -convergence (Conjecture 3.1) implies also the convergence of the discrete minimisers to a continuum minimiser of the limit functional. Since we are interested in the deformations of the cell culture, this is the central result, rather than the convergence of energies.

Conjecture 3.1 considers an unconstrained Γ -limit, i.e. no boundary conditions are considered. However, often one is interested in problems with prescribed boundary conditions. Corresponding convergence could be proven. Similar results hold also for periodic boundary conditions. For more details on the proof of Conjecture 3.1 as well as on the corollarys considering different boundary conditions, we refer to the work of Alicandro and Cicalese [17].

3.2. Homogenisation formula

Conjecture 3.1 states the existence of a continuum limit for Model 2.1. However, it does not state how such a continuum limit looks like. Using the symmetry of the underlying geometry, it is possible to derive an explicit representation formula.

Conjecture 3.2. For all times t and for every sequence (ε_j) of positive real numbers converging to 0, the sequence $(E^{\varepsilon_j}(t))$ given in (9) Γ -converges with respect to the $L^2(\Omega; \mathbb{R}^2)$ -topology to $E(t) : L^2(\Omega; \mathbb{R}^2) \to [0, \infty]$ defined as

$$E(\boldsymbol{\chi}(t),t) \equiv \begin{cases} \int_{\Omega} J^{G}(t) \Psi(\nabla_{X} \boldsymbol{\chi}(t),t) d\boldsymbol{X} & \text{if } \boldsymbol{\chi}(t) \in W^{1,2}(\Omega;\mathbb{R}^{2}) \\ \infty & \text{otherwise,} \end{cases}$$

where the integrand $\Psi: \mathbb{R}^{2 \times 2} \to [0, \infty)$ is given by the following problem on a unit cell

(10)

$$\Psi(\nabla_X \boldsymbol{\chi}(t), t) \equiv E_{perimeter} \left(\sum_{\alpha \in \mathcal{T}_0^{\varepsilon=1}} |\nabla_X \boldsymbol{\chi}(t) \cdot \boldsymbol{\zeta}_{\alpha, \alpha+1}|, P_0(t) \right) \\
+ \sum_{j \in links} E_{link} \left(|\nabla_X \boldsymbol{\chi}(t) \cdot \boldsymbol{\xi}_{0,j}|, L_{0,j}(t) \right)$$

with vectors $\boldsymbol{\zeta}_{\alpha,\alpha+1}$ and $\boldsymbol{\xi}_{0,j}$ corresponding to the undeformed unit cell denoted with the index 0 (cf. Fig. 2).

As outlined above, Conjecture 3.2 implies that the minimisers of the discrete energy E^{ε} given in Model 2.1 converge to minimisers of the continuum energy E for $\varepsilon \to 0$. Thus solutions of the following macroscopic continuum model approximate solutions of the microscopic discrete Model 2.1.

Model 3.1. For any given time t, the quasi-stationary shape / deformation $\chi(t)$ of the culture Ω is given by the minimiser of the following macroscopic continuum energy

$$E(\boldsymbol{\chi}(t),t) \equiv \begin{cases} \int_{\Omega} J^{G}(t) \Psi(\nabla_{X} \boldsymbol{\chi}(t),t) d\boldsymbol{X} & \text{if } \boldsymbol{\chi}(t) \in W^{1,2}(\Omega;\mathbb{R}^{2}) \\ \infty & \text{otherwise,} \end{cases}$$

with the energy density $\Psi(\nabla_X \chi(t), t)$ specified in Conjecture 3.2.

That is, Model 3.1 is the macroscopic continuum counterpart / macroscopic approximation of the microscopic discrete Model 2.1.

4. Macroscopic continuum mechanical models

The models considered above, i.e. Model 2.1 and Model 3.1, are based on a static description in terms of energy functionals: shapes of tissues are determined by the energy minimising configurations. The energy minima can be calculated using the corresponding Euler-Lagrange equations [25]. Physically speaking, the corresponding forces of the energies are determined and one looks for a shape where all forces equilibrate.

Instead of using the Euler-Lagrange equations, a relaxation approach based on a dynamic formulation in the framework of conservation of mass and linear momentum can be considered equivalently. The stress tensor, i.e. surface force densities, can be obtained directly from variational principles (in analogy to the Euler-Lagrange equations) on the basis of the multiscale energy density Ψ derived above. Since this approach corresponds to the derivation of the Euler-Lagrange equations, stationary states of Model 4.1 are minimisers of the continuum version of Model 2.1, i.e. Model 3.1. Such a relaxation approach is of course somewhat more complex, but has several advantages: it can be easily extended to situations, where dynamics play a role, as well as it can be easily compared with existing continuum models, e.g. [7–15].

Model 4.1. The evolution of the cell culture $\Omega(t)$ with $\Omega(0) \equiv \Omega$ is determined by the following set of equations (for some fixed time T > 0)

$$\begin{aligned} \frac{d}{dt} \boldsymbol{\chi} &= \boldsymbol{v} & \text{in } \Omega(t) \times [0, T), \\ \frac{d}{dt} \rho &= \rho \text{tr} \mathbf{D}_G & \text{in } \Omega(t) \times [0, T), \\ \frac{d}{dt} (\rho \boldsymbol{v}) &= \nabla_x \cdot \sigma(\mathbf{F}, \mathbf{G}) + \boldsymbol{v} \rho \text{tr} \mathbf{D}_G & \text{in } \Omega(t) \times [0, T), \\ \frac{d}{dt} \mathbf{G} &= \mathbf{g} & \text{in } \Omega(t) \times [0, T), \end{aligned}$$

with appropriate initial and boundary conditions.

Here, $\frac{d}{dt}\rho \equiv \frac{\partial}{\partial t}\rho + \nabla_x \cdot (\rho \boldsymbol{v})$ is the material derivative and ∇_x the derivative with respect to Eulerian coordinates. The constant ρ is the material density, $\boldsymbol{\chi}$ the material deformation, \boldsymbol{v} the material speed, and $\sigma(\mathbf{F}, \mathbf{G})$ the stress tensor. The latter can be directly computed from the Euler-Lagrange equations of Model 3.1, as shown below. The stress tensor depends on the deformation tensor $\mathbf{F} = \nabla_x \boldsymbol{\chi}$ and the growth tensor \mathbf{G} with

 $\mathbf{D}_G = \frac{1}{2} (\frac{d}{dt} \mathbf{G} \cdot \mathbf{G}^{-1})^T + (\frac{d}{dt} \mathbf{G} \cdot \mathbf{G}^{-1})$ and growth rate **g**. Above, we have implicitly assumed that growth also implies the generation of momentum via mass growth (cf. the source term $\rho \boldsymbol{v} \operatorname{tr} \mathbf{D}_G$ in the linear momentum balance), which is a typical assumption considering growing cell cultures [13,14].

4.1. Derivation of the stress tensor σ

Using a variational approach, i.e. computing the corresponding Euler-Lagrange equations of the continuum model, we can relate the energies derived in Section 3 with corresponding forces. The forces are given by the steepest decent of the L^2 -gradient of the free energy. To derive the steepest decent of the L^2 -gradient (i.e. the Fréchet derivative [24]), let us consider small variations $\chi_{\epsilon} = \chi + \epsilon \phi$, where $\phi \in C^{\infty}(\mathbb{R}^2; \mathbb{R}^2)$ is an arbitrary test function. Using $\frac{d}{d\epsilon} \mathbf{F}(t)|_{\epsilon=0} = \nabla_X \phi = (\nabla_x \phi) \cdot \mathbf{F}(t)$, we find

where $\mathcal{P}_0(t) = \mathcal{P}_0(\mathbf{F}(t)), P_0(t) = P_0(\mathbf{G}(t)), \mathcal{L}_{0,j}(t) = \mathcal{L}_{0,j}(\mathbf{F}(t)),$ and $L_{0,j}(t) = L_{0,j}(\mathbf{G}(t)).$ Here, $\hat{\boldsymbol{\xi}}(t) = \mathbf{F}(t) \cdot \boldsymbol{\xi}$ and $\hat{\boldsymbol{\zeta}}(t) = \mathbf{F}(t) \cdot \boldsymbol{\zeta}$ are the vectors $\boldsymbol{\xi}$ and $\boldsymbol{\zeta}$ in the deformed configuration. Using $\int_{\Omega} (\nabla_x \cdot \sigma) \cdot \phi d\boldsymbol{x} = \frac{d}{d\epsilon} E|_{\epsilon=0}$ the stress tensor σ is recovered:

(11)

$$\sigma(\mathbf{F}, \mathbf{G}) = \frac{\det \mathbf{G}}{\det \mathbf{F}} \left(\frac{\partial}{\partial \mathcal{P}_0} E_{\text{perimeter}} \left(\mathcal{P}_0(\mathbf{F}), P_0(\mathbf{G}) \right) \\ \sum_{\alpha \in \mathcal{T}_0^1} \frac{1}{2\mathcal{L}_{\alpha,\alpha+1}(\mathbf{F})} (\mathbf{F} \cdot \boldsymbol{\zeta}_{\alpha,\alpha+1}) \otimes (\mathbf{F} \cdot \boldsymbol{\zeta}_{\alpha,\alpha+1}) \\ + \sum_{j \in \text{links}} \frac{\partial}{\partial \mathcal{L}_{0,j}} E_{\text{link}} \left(\mathcal{L}_{0,j}(\mathbf{F}), L_{0,j}(\mathbf{G}) \right) \frac{1}{2\mathcal{L}_{0,j}(\mathbf{F})} \\ \left(\mathbf{F} \cdot (\boldsymbol{\xi}_{0,j}) \otimes (\mathbf{F} \cdot \boldsymbol{\xi}_{0,j}) \right),$$

where boundary terms have been neglected assuming appropriate boundary conditions. Above we have formulated σ in terms of the deformation tensor **F** and the growth tensor **G**. Using $\mathbf{F} = \mathbf{F}^{\text{mech}} \cdot \mathbf{G}$ and thus det $\mathbf{G}/\det \mathbf{F} = 1/\det \mathbf{F}^{\text{mech}}$, a formulation of σ in terms of the mechanical deformation tensor \mathbf{F}^{mech} and the growth tensor **G** follows directly along the lines of Section 2.3.1, i.e. we find $\sigma(\mathbf{F}, \mathbf{G}) = \sigma(\mathbf{F}^{\text{mech}})$.

4.2. Linear elasticity

Mechanical properties of the macroscopic continuum mechanical model (Model 4.1) are directly related to the microscopic properties of the idealised single cells. Thus, in the isotropic case we can relate the Lamé constants μ and λ , used in linear elasticity, directly with the properties of the discrete atomistic model. Considering small deformations and restricting ourselves to linear elasticity, i.e. performing a Taylor extension considering only linear terms, we recover from equation (11)

$$\mu = \frac{3}{2}\kappa_L,$$
$$\lambda = \frac{3}{2}\kappa_L + \kappa_P$$

5. Discussion and Outlook

The presented approach offers the possibility to understand mechanobiological phenomena in a truly multiscale manner resolving the conflict between the level of detail and computational complexity. Based on such a multiscale approach it is e.g. possible to derive highly realistic models for growing cell cultures, a fundamental system in developmental biology.

In this work, we have shown how concepts from Γ -convergence, introduced for the derivation of continuum macroscopic models considering atomistic interactions in crystal lattices [17], could also be applied to active biomechanical systems, namely growing tissues. The analysis is based on a simplified discrete microscopic model in terms of energy functionals, which can be related to a large class of discrete models studying growing tissues [1–6]. Following the ideas of [17], we have then outlined how to derive from the discrete microscopic model a corresponding macroscopic continuum energy functional for growing tissues using the concept of Γ convergence. Via a homogenisation formula microscopic details are taken explicitly into account. The properties of Γ -convergence imply that energy minimising configurations of the detailed microscopic model are approxi-

mated by the ones of the macroscopic model. The latter ones can be calculated via the corresponding Euler Lagrange equations directly or via a relaxation approach leading to a continuum model in the framework of balance equations (balance of mass and linear momentum), typically used in continuum approaches to growing tissues and cell cultures [7–15].

Although the discrete model could be considered as the more realistic one, since it includes all the microscopic details, a continuum description is often preferable. On the one hand it is more accessible by mathematical analysis and on the other hand computations can be based on "arbitrary" coarse discretisations (saving computational effort) rather than the given microscopic topologies. Our approach guarantees that the derived continuum approximation is always sufficiently close to the microscopic discrete description. Within the required accuracy it can be substituted for the discrete model.

In the case of isotropic growth, our approach coincides with the notion of multiple natural configurations [7,20] used in many continuum approaches [7–15]. It postulates a multiplicative decomposition of the deformation gradient into a deformation related to mechanics and one related to growth. However, in the case of anisotropic growth our approach postulates that remodelling has to be considered, i.e. an evolution of the underlying stress-strain relationships (constitutive equations). Approaches using multiple natural configurations directly usually neglect remodelling since evolution laws for the stress tensor are not obvious. Our approach provides appropriate evolution laws for the stress tensor via explicit homogenisation formulae allowing a more realistic modelling of anisotropic growing cell cultures, e.g. muscles or cultures of rod shaped bacteria.

Deriving the continuum model, we have relied on a number of assumptions for the sake of mathematical rigorousness. A detailed and careful study how these assumptions could be lifted is an important part of future work. So far, the approach could lift the assumption of a hexagonal symmetry in the underlying cell culture (which is necessary for the derivation of appropriate homogenisation formulae) via heuristic averaging. More rigorous approaches should be investigated. Furthermore, we have restricted ourselves to solely elastic cell cultures as in many other approaches [13,14]. Since, experimental findings suggest that a Maxwell visco-elastic description could be more appropriate in some tissues / cultures [26], an extension of the ideas presented here has to be investigated. A sketch of a formal approach can be found in [27].

Within this work, we have restricted ourselves to a simplified model of existing discrete microscopic models of tissues and cell cultures [1,3–6] without a specific application in mind. Experimental results concerning microscopic details of exact cell structures in growing tissues as necessary for the proposed multiscale approach are lacking. Since imaging techniques are advancing extremely fast this information should be available in the near future. In combination with detailed microscopic measurements of mechanical moduli of microscopic sub-elements our approach would provide detailed quantitative models for tissues and cell cultures. These are extremely difficult to obtain by experimental means alone. We believe that our multiscale approach is also very promising with respect to the derivation of appropriate macroscopic models based on microscopic details in many other biological applications where reorganisation or growth of sub-cellular elements play an important role, e.g. in migrating keratocytes [28].

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