Multiphase Models of Tumour Growth

S. Astanin and L. Preziosi

Dipartimento di Matematica, Politecnico di Torino,
corso Duca degli Abruzzi 24, 10129 Torino, Italy
{sergey.astanin, luigi.preziosi}@polito.it

Summary. The aim of this chapter is to give a pedagogical presentation of multiphase models in their application to the study of tumour growth. Starting from the simplest concepts, we shall describe how to deduce multiphase models, paying attention to the general modelling framework and on how to model the different terms appearing in the equations. A particular attention is also devoted to the definition of the interaction between cells and extracellular matrix. In this way a general model is deduced which is then specialized in examples describing avascular phase and vascular phases of growth, and the formation of fibrosis.

1 Introduction

Mixture theory has been applied to describe the mechanics of biological tissues since the sixties. Most of the work was focused on the behaviour of articular cartilages [24, 29, 30, 34, 35, 36, 37], but applications can be found to many soft tissues, e.g., brain [39, 47], heart mechanics [49, 53, 54], subcutaneous layer [40], flow through arteries [26, 27, 28].

In the last few years mixture theory has been also applied with success to tumour growth. Examples of applications can be found in [10, 11, 12, 13, 16, 18, 19, 20] while [3, 5, 23] are review papers on this approach and on the mechanical aspects related to tumour growth. Here, we shall deduce a general multiphase modelling framework for few but essential constituents (cells, extracellular matrix, and extracellular liquid with the solutes dissolved in it). We shall also show how to take into account several sub-populations of the cells, and several components of the extracellular matrix (ECM).

There are three basic hypotheses that allow to obtain a manageable model even in the case of more constituents involved. The first hypothesis is an assumption that the components of the extracellular matrix form such an intricate network that they all move together so that the same deformation and velocity describe their evolution. The second one consists in assuming that the pressure gradient and the interaction forces involving the liquid are

much smaller than the others, e.g., the adhesion force between cells and extracellular matrix. The third one consists in assuming that cells mechanically respond to the compression coming from the surrounding cells in the same way independently from the cell type.

All the steps of the modelling procedure are explained in detail, special attention being paid to the meaning of all the different terms involved in the model. Some examples are given to clarify how to model them. Specifically, in Section 2 we deal with mass balance equations, in Section 3 with force balance equations. Section 4 is in large part devoted to the description of the interaction between the cells and the extracellular matrix. In Section 5 we address the issue of how to deduce a proper constitutive model for the stress, though the reader is referred to more specific literature for further details [3, 4, 5, 23]. Finally, in the last three sections, the general model is specialized to describe tumour growth in an immutabile ECM (rigid, non-remodelling), including the mechanical interaction with the host tissue, the growth in a rigid remodelling ECM with the aim of showing the formation of fibrosis, and the growth of a vascularized tumour, with a particular focus on how to relate cell metabolism to growth terms.

2 Mass Balance

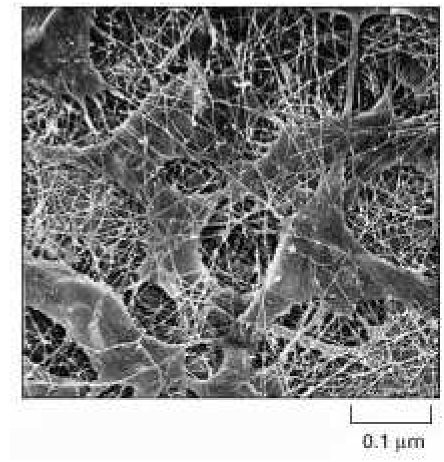
Soft tissues are mainly made of cells and extracellular matrix (ECM). The porous material that this ensemble forms is wet by an extracellular liquid full of chemicals: nutrients, growth factors, chemotactic factors, and so on (see Fig. 1a).

Of course, the typical size of cells and ECM composed of aggregated proteins is much bigger than that of dissolved proteins. So, one can assume that the space occupied by the latter is negligible and one can treat them as part of the extracellular liquid.

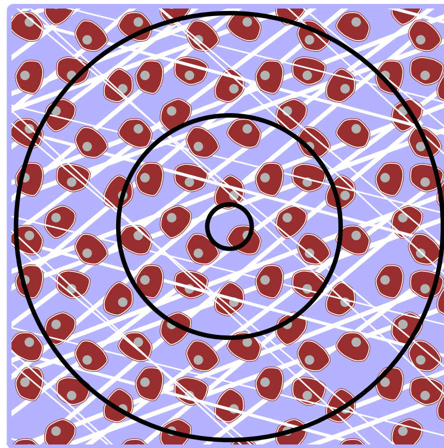
For all the constituents of the mixture we define their volume ratios as follows. Given a point in the mixture consider a sequence of spheres with the centres at the point (Fig. 1b). Measuring the ratio of the volume of a given constituent inside the sphere to the volume of the sphere one may observe the dependency shown in Fig. 1c. For small sample volumes the ratio is likely to oscillate due to microscopic inhomogenity. Macroscopic inhomogenities may affect the ratio for large sample volumes. However for sample volumes in between, at scales larger than the cell size and smaller than typical tissue scale, it is nearly constant and allows us to define a quantity called volume ratio of the constituent.

Let us denote by $\phi \in [0,1]$ and $m \in [0,1]$ the volume ratios occupied by cells and by the extracellular matrix respectively. The mixture is *saturated* if the rest of the space is filled by extracellular liquid $\ell \in [0,1]$, i.e.,

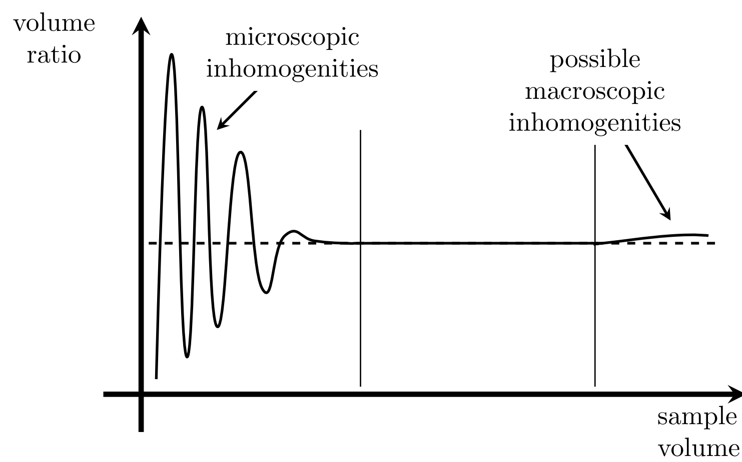
$$\phi + m + \ell = 1\,. \tag{1}$$



(a)



(b)



(c)

Fig. 1. (a) Tissue with fibroblasts and extracellular matrix. Three sample volumes are shown as black circles in (b), ECM in white, cells in darker tint, the rest is extracellular liquid. (c) Volume ratio of the constituent as a function of sample volume size.

In some models the upper constraint on the volume ratio is replaced by a constant value $\overline{\phi} < 1$, possibly space dependent, allowing for some fixed portion of space to be occupied by other constituents not considered in the mixture, e.g., vessels or a general stroma. In this case the saturation constraint is replaced by

$$\phi + m + \ell = \overline{\phi}\,, \tag{2}$$

with all volume ratios in $[0, \overline{\phi}]$.

If $\overline{\phi}$ is space independent, it is possible to rescale the variables as $\tilde{\phi} := \phi/\overline{\phi}$, $\tilde{m} := m/\overline{m}$, $\tilde{l} := l/\overline{l}$ to have $\tilde{\phi} + \tilde{m} + \tilde{l} = 1$.

In order to define the mass balance equations for the different constituents consider a general volume $\mathcal{V}$ fixed in space with boundary $\partial\mathcal{V}$. To be specific we shall focus on the cellular constituent of the tissue. If ρ is the density within the cells, the mass of the constituent in $\mathcal{V}$

$$\int_{\mathcal{V}} \rho\phi\, dV$$

can change due to:

1. Flux caused by the motion of the constituent through the boundary $\partial\mathcal{V}$

$$-\int_{\partial\mathcal{V}} \rho\phi \mathbf{v}_\phi \cdot \mathbf{n}\, d\Sigma\,,$$

where $\mathbf{v}_\alpha$ is the cell velocity and $\mathbf{n}$ is an external normal to the boundary $\partial\mathcal{V}$;

2. Growth or death of cells

$$\int_{\mathcal{V}} \rho\Gamma_\phi\, dV\,,$$

where Γ_ϕ is the mass exchange rate or growth/death rate for the cellular mass.

One then has

$$\frac{d}{dt}\int_{\mathcal{V}} \rho\phi\, dV = -\int_{\partial\mathcal{V}} \rho\phi\mathbf{v}_\phi \cdot \mathbf{n}\, d\Sigma + \int_{\mathcal{V}} \rho\Gamma_\phi\, dV\,.$$

Using Gauss theorem, one can write

$$\int_{\mathcal{V}} \left[\frac{\partial}{\partial t}(\rho\phi) + \nabla\cdot(\rho\phi\mathbf{v}_\phi) - \rho\Gamma_\phi\right] dV = 0\,,$$

and, if the integrands are smooth, because of the arbitrariety of the volume of integration $\mathcal{V}$,

$$\frac{\partial}{\partial t}(\rho\phi) + \nabla\cdot(\rho\phi\mathbf{v}_\phi) = \rho\Gamma_\phi\,. \tag{3}$$

where ρ can be taken constant and equal to the density of water.

Generalizing the procedure for all the variables mentioned above one can write the mass balance equations

$$\begin{aligned}
&\frac{\partial \phi}{\partial t} + \nabla \cdot (\phi \mathbf{v}_\phi) = \varGamma_\phi \,, \\
&\frac{\partial m}{\partial t} + \nabla \cdot (m \mathbf{v}_m) = \varGamma_m \,, \\
&\frac{\partial \ell}{\partial t} + \nabla \cdot (\ell \mathbf{v}_\ell) = \varGamma_\ell \,.
\end{aligned} \tag{4}$$

If the mixture is *closed* so that mass exchange occurs only between the constituents taken into consideration then

$$\varGamma_\phi + \varGamma_m + \varGamma_\ell = 0 \,, \tag{5}$$

where for the sake of simplicity we assumed that all constituents of the tissue have equal density ρ, the density of the extracellular liquid.

In other case, when external mass sources/sinks are introduced to describe outflow/inflow processes related, for example, to a homogenised vascular or lymphatic structure, the condition (5) might be dropped. This approach is, for instance, used in [18, 19, 20]. However, also in this case one needs to assure that the solution never violates the geometrical constraint (1) or (2) during the evolution.

According to the details needed to describe the phenomenon of interest, it may be necessary to distinguish different cell populations, e.g., tumour cells, endothelial cells, epithelial cells, fibroblasts, macrophages, lymphocytes, or to distinguish different clones within the same population characterized by relevant differences in their behaviour, or to distinguish the cells according to their phase in the cell cycle, e.g. G_0, G_1, G_2.

If this is the case, the first equation in (4) must be split in I equations, one for each of the I subpopulations:

$$\frac{\partial \phi_i}{\partial t} + \nabla \cdot (\phi_i \mathbf{v}_{\phi_i}) = \varGamma_{\phi_i} \,, \qquad i = 1, \ldots, I \,, \tag{6}$$

where ϕ_i is the volume ratio of the subpopulation i, $\mathbf{v}_i$ is its velocity and $\varGamma_{\phi_i}$ is its mass exchange rate. Of course,

$$\sum_{i=1}^{I} \phi_i = \phi \,, \qquad \sum_{i=1}^{I} \phi_i \mathbf{v}_{\phi_i} = \phi \mathbf{v}_\phi \,, \qquad \sum_{i=1}^{I} \varGamma_{\phi_i} = \varGamma_\phi \,, \tag{7}$$

and therefore summing all (6) over i gives back the first equation in (4).

Similarly, because of the different mechanical behaviour and chemical properties, it might be necessary to distinguish the different components of

the ECM, e.g. collagen, elastin, fibronectin, vitronectin, proteoglycans. One then has

$$\frac{\partial m_j}{\partial t} + \nabla \cdot (m_j \mathbf{v}_m) = \Gamma_{m_j} \,, \qquad j = 1, \ldots, J \,, \tag{8}$$

where m_j is the volume ratio of the j-th component and Γ_{m_j} is its remodelling rate. We explicitly notice that in (8) the ECM velocity is taken to be the same for all ECM components, which means describing them as an intricate network of fibres that have to move all together. This is called a *constrained sub-mixture assumption*. As before

$$\sum_{j=1}^{J} m_j = m \,, \qquad \sum_{j=1}^{J} \Gamma_{m_j} = \Gamma_m \,, \tag{9}$$

and summing (8) over j gives the second equation in (4).

Other fundamental factors influencing tumour evolution are the various proteins and chemicals that govern the growth and the behaviour of the cells. One can treat them as solutes dispersed in the extracellular liquid, transported and diffusing with it. Their concentrations are the quantities of interest from the modelling point of view.

For the sake of simplicity, consider some chemical and denote by c_α its concentration per unit volume within the constituent α of the mixture, where for instance α might be ϕ, ℓ, or m. However, the concentration c_α has to be related to the volume ratio occupied by the constituent in which it is present, so that finally the relevant entities for an overall balance over the whole mixture are the *reduced* (or *weighted*) *concentrations*, e.g., $C_\ell = \ell c_\ell$.

To be specific consider the diffusion in the liquid constituent. In order to compute its balance we have to consider the motion of the fluid, the diffusive flux, the absorption of the liquid in which the chemical is dissolved and the absorption of the chemical without absorption of the liquid, e.g. by osmosis.

One then has the following integral balance equation

$$\frac{d}{dt}\int_{\mathcal{V}} \ell c_\ell \, dV = -\int_{\partial\mathcal{V}} \ell c_\ell \mathbf{v}_\ell \cdot \mathbf{n} \, d\Sigma - \int_{\partial\mathcal{V}} \ell \mathbf{j}_\ell \cdot \mathbf{n} \, d\Sigma + \int_{\mathcal{V}} \Gamma_\ell c_\ell \, dV + \int_{\mathcal{V}} G_c \, dV \,, \tag{10}$$

where $\mathbf{j}_\ell$ is diffusive flux inside liquid

$$\mathbf{j}_\ell = D_\ell \nabla c_\ell$$

and Γ_c is the chemical exchange rate (rate of production/uptake). Taking for instance Γ_ℓ negative, the term $\int_{\mathcal{V}} \Gamma_\ell c_\ell \, dV$ reflects adoption of the chemical by other constituents of the mixture through the means of capturing the liquid.

Therefore the following reaction-convection-diffusion equation can be deduced

$$\frac{\partial}{\partial t}(\ell c_\ell) + \nabla \cdot (\ell c_\ell \mathbf{v}_\ell) = \nabla \cdot (\ell \mathbf{j}_\ell) + G_c + \Gamma_\ell c_\ell \,. \tag{11}$$

This equation can be written in terms of $C_\ell = \ell c_\ell$, but Fick's law states that the diffusive flux can be assumed to be proportional to the concentration gradient in the liquid, that is

$$\mathbf{j}_\ell = \mathbf{D}_\ell \nabla c_\ell = \mathbf{D}_\ell \nabla \frac{C_\ell}{\ell}\,, \tag{12}$$

where $\mathbf{D}_\ell = \mathbf{D}_\ell(\phi_\ell)$ is the effective diffusion tensor in the liquid which accounts for diffusion of the chemical in the liquid due to Brownian motion as well as for molecules dispersion due to the porous structure of the mixture (see [8]). Hence, one has

$$\frac{\partial C_\ell}{\partial t} + \nabla \cdot (C_\ell \mathbf{v}_\ell) = \nabla \cdot \left(\ell \mathbf{D}_\ell \nabla \frac{C_\ell}{\ell} \right) + G_c + \Gamma_\ell \frac{C_\ell}{\ell}\,. \tag{13}$$

On the other hand, using the mass balance equation for the liquid $(4)_3$, Eq. (11) simplifies to

$$\ell \left(\frac{\partial c_\ell}{\partial t} + \mathbf{v}_\ell \cdot \nabla c_\ell \right) = \nabla \cdot (\ell \mathbf{D}_\ell \nabla c_\ell) + G_c\,. \tag{14}$$

Similar equations can be written for the concentration of chemicals in the other constituents. However, if exchange of the chemical between the constituents is so fast that one might assume that concentration of the chemical is the same for all the constituents:

$$c = c_l = c_\phi = c_m,$$

then the summation gives

$$\frac{\partial c}{\partial t} + \mathbf{v}_c \cdot \nabla c = \nabla \cdot (\mathbf{D} \nabla c) + G\,, \tag{15}$$

where $\mathbf{D}$ is an effective diffusivity tensor in the mixture, G contains production/source terms and degradation/uptake terms relative to the entire mixture and $\mathbf{v}_c = \phi \mathbf{v}_\phi + \ell \mathbf{v}_\ell + m \mathbf{v}_m$ is the composite velocity. Actually, the related convective term can be neglected in most applications, so that one can write

$$\frac{\partial c}{\partial t} = \nabla \cdot (\mathbf{D} \nabla c) + G\,. \tag{16}$$

The procedure above can be generalized to all soluble molecules which are considered relevant to tumour development.

3 Force Balance

Several methods have been used to close the system of mass balance equations introduced in the previous section (the reader is referred to [3] for a critical

review on this aspect). We here focus on the use of momentum balance equations, which we start writing in integral form for a generic constituent for pedagogical reasons to clarify the origin of all terms appearing in the equations.

Focusing again on the cellular constituent of the tissue the variation of momentum of the constituent in the fixed volume $\mathcal{V}$

$$\int_{\mathcal{V}} \rho\phi\mathbf{v}_\phi \, dV$$

is due to

1. Momentum flux caused by the motion of the cells through the boundary $\partial\mathcal{V}$
$$-\int_{\partial\mathcal{V}} \rho\phi\mathbf{v}_\phi(\mathbf{v}_\phi \cdot \mathbf{n}) \, d\Sigma \,;$$
2. Contact forces within the constituent acting through the boundary $\partial\mathcal{V}$, which are codirected with $\mathbf{n}$, therefore yielding
$$\int_{\partial\mathcal{V}} \widetilde{\mathbf{T}}_\phi^T \mathbf{n} \, d\Sigma \,,$$
where $\widetilde{\mathbf{T}}_\phi$ is called partial stress;
3. Contact forces due to the interaction with the other constituents within the domain through the interface separating the constituents, say the cell membrane wet by the extracellular liquid or in contact with the extracellular matrix through the adhesion sites
$$\int_{\mathcal{V}} \widetilde{\mathbf{m}}_\phi \, dV \,,$$
where $\widetilde{\mathbf{m}}_\phi$ is called interaction force;
4. Momentum supply related to phase changes
$$\int_{\mathcal{V}} \rho\Gamma_\phi \mathbf{v}_\phi \, dV \,,$$
e.g., fluid absorbed by a growing cell, ECM production or degradation;
5. Body forces
$$\int_{\mathcal{V}} \rho\phi\mathbf{b} \, dv \,,$$
e.g., chemotaxis or haptotaxis can be modelled in this way, though they actually involve the activation of sub-cellular mechanisms rather than an external action.

One then has

$$\frac{d}{dt}\int_{\mathcal{V}} \rho\phi\mathbf{v}_\phi \, dV = \int_{\partial\mathcal{V}} \left[-\rho\phi\mathbf{v}_\phi(\mathbf{v}_\phi \cdot \mathbf{n}) + \widetilde{\mathbf{T}}_\phi^T \mathbf{n}\right] \, d\Sigma + \int_{\mathcal{V}} (\rho\phi\mathbf{b} + \widetilde{\mathbf{m}}_\phi + \rho\Gamma_\phi \mathbf{v}_\phi) \, dV \,. \tag{17}$$

So using Gauss theorem we can write

$$\int_{\mathcal{V}} \left[\frac{\partial}{\partial t}(\rho\phi\mathbf{v}_\phi) + \nabla\cdot(\rho\phi\mathbf{v}_\phi\otimes\mathbf{v}_\phi) - \nabla\cdot\widetilde{\mathbf{T}}_\phi - \rho\phi\mathbf{b} - \widetilde{\mathbf{m}}_\phi - \rho\Gamma_\phi\mathbf{v}_\phi\right] dV = 0\,. \tag{18}$$

This holds for any volume of integration $\mathcal{V}$. So, if the integrand is smooth, one can write the following local form of the momentum balance for the solid constituent in conservative form

$$\frac{\partial}{\partial t}(\rho\phi\mathbf{v}_\phi) + \nabla\cdot(\rho\phi\mathbf{v}_\phi\otimes\mathbf{v}_\phi) = \nabla\cdot\widetilde{\mathbf{T}}_\phi + \rho\phi\mathbf{b} + \widetilde{\mathbf{m}}_\phi + \rho\Gamma_\phi\mathbf{v}_\phi\,. \tag{19}$$

Actually using the mass balance equation $(4)_1$, Eq. (19) can be simplified as

$$\rho\phi\left(\frac{\partial\mathbf{v}_\phi}{\partial t} + \mathbf{v}_\phi\cdot\nabla\mathbf{v}_\phi\right) = \nabla\cdot\widetilde{\mathbf{T}}_\phi + \rho\phi\mathbf{b} + \widetilde{\mathbf{m}}_\phi\,, \tag{20}$$

where the inertial term on the left hand side can be usually neglected when describing biological growth phenomena.

If a saturation condition like (1) is assumed, then the constitutive equations for the partial stresses and for the interaction forces are characterized by the presence of a Lagrange multiplier classically identified with the interstitial pressure of the extracellular liquid [9, 45]. Without going into technical details, this is related to the fact that in checking the validity of the second principle of thermodynamics, one is considering only all those processes satisfying the saturation constraint. The presence of a constraint implies the need of introducing a Lagrange multiplier in Clausius–Duhem inequality, so that it is considered for any process such that the saturation constraint holds.

A similar reasoning is usually done in fluid dynamics where enforcing incompressibility implies that one is studying only flows satisfying such a constraint and for this class of processes the second principle of thermodynamics should hold. The consequence is that in the constitutive equation for the fluid, the isotropic part of the stress tensor can not be determined constitutively but is a reaction that adjusts so that the incompressibility constraint is satisfied.

In the case of the saturated mixture, for instance, it can be proved that

$$\widetilde{\mathbf{T}}_\phi = -\phi P\mathbf{I} + \phi\mathbf{T}_\phi\,, \qquad \widetilde{\mathbf{m}}_\phi = P\nabla\phi + \mathbf{m}_\phi\,, \tag{21}$$

where $\mathbf{T}_\phi$ is called excess stress and $\mathbf{m}_\phi$ excess interaction force.

One then has

$$-\phi\nabla P + \nabla\cdot(\phi\mathbf{T}_\phi) + \mathbf{m}_\phi + \rho\phi\mathbf{b} = \mathbf{0}\,. \tag{22}$$

Proceeding in a similar way for the other constituents and specifying, if needed, the different force balance equations for the cellular components one can write

$$
\begin{aligned}
&-\phi_i\nabla P + \nabla\cdot(\phi_i \mathbf{T}_{\phi_i}) + \mathbf{m}_{\phi_i} + \rho\phi_i \mathbf{b}_i = \mathbf{0}\,,\\
&-m\nabla P + \nabla\cdot(m\mathbf{T}_m) + \mathbf{m}_m = \mathbf{0}\,, \qquad (23)\\
&-\ell\nabla P + \mathbf{m}_\ell = \mathbf{0}\,,
\end{aligned}
$$

where the excess stress tensor for the extracellular liquid is assumed to be negligible, as it is usually done in the theory of deformable porous media to obtain Darcy's law like behaviour, and the body forces are dropped in the equations for the ECM and for the liquid [9, 45].

We observe explicitly, that even if several ECM components need be specified, the constrained sub-mixture hypothesis allows to write a single force balance equation to determine the common velocity $\mathbf{v}_m$. Of course, all the ECM components will contribute to the constitutive equation for the stress tensor according to their relative proportion.

4 Interaction Forces

If the mixture is closed, one may demonstrate that in mixture theory the sum of interaction forces and of the momentum transfers due to mass exchange is zero [9, 45]. However, the contribution due to mass exchange is negligible with respect to that due to the interaction forces [42], compatibly with the fact that inertial terms are negligible. Hence, one can say that the interaction forces sum up to zero, as it might be expected since they act as internal forces among the constituents of the whole mixture.

We reinforce this concept of internal forces by assuming that if the constituent β exerts an interaction force $\mathbf{m}_{\alpha\beta}$ on the constituent α, in turn the constituent α will exert on β an equal and opposite force, i.e., $\mathbf{m}_{\alpha\beta} = -\mathbf{m}_{\beta\alpha}$, being aware of the fact that this equality is an approximation, for instance, for the presence of exchanges of mass.

We distinguish among the interaction forces those involving the extracellular liquid, because they can be treated as drag forces. The others might require better understanding of the adhesion mechanisms.

Compatibly with Darcy's law, the interaction forces of all the constituents with the liquid can be taken to be proportional to the velocity difference between the liquid and the other constituents through invertible matrices $\mathbf{M}_i$, and $\mathbf{M}_m$, so that

$$
\begin{aligned}
\mathbf{m}_{\ell\phi_i} &= -\mathbf{M}_i(\mathbf{v}_\ell - \mathbf{v}_{\phi_i})\,,\\
\mathbf{m}_{\ell m} &= -\mathbf{M}_m(\mathbf{v}_\ell - \mathbf{v}_m)\,,
\end{aligned} \qquad (24)
$$

where $\mathbf{M}_i$ and $\mathbf{M}_m$ are invertible matrices. Therefore, in the last equation of (23) $\mathbf{m}_\ell = \mathbf{m}_{\ell m} + \sum_i \mathbf{m}_{\ell\phi_i}$. One then has

$$
\mathbf{M}_m(\mathbf{v}_\ell - \mathbf{v}_m) + \sum_{i=1}^{I} \mathbf{M}_i(\mathbf{v}_\ell - \mathbf{v}_{\phi_i}) = -\ell\nabla P\,, \qquad (25)
$$

or

$$\mathbf{v}_\ell = \mathbf{M}^{-1}\left(\mathbf{M}_m\mathbf{v}_m + \sum_{i=1}^{I}\mathbf{M}_i\mathbf{v}_{\phi_i} - \ell\nabla P\right), \tag{26}$$

where $\mathbf{M} = \mathbf{M}_m + \sum_i \mathbf{M}_i$, which explicitly gives the liquid velocity in terms of the other velocities and of the pressure gradient.

Furthermore, summing the mass balance equations (4) one has for a closed mixture

$$\nabla\cdot\left(\ell\mathbf{v}_\ell + m\mathbf{v}_m + \sum_{i=1}^{I}\phi_i\mathbf{v}_{\phi_i}\right) = 0, \tag{27}$$

which substituting the velocity of the liquid reduces to

$$\nabla\cdot(\ell^2\mathbf{M}^{-1}\nabla P) = \nabla\cdot\left[\sum_{i=1}^{I}(\phi_i\mathbf{I} + \ell\mathbf{M}^{-1}\mathbf{M}_i)\mathbf{v}_{\phi_i} + (m\mathbf{I} + \ell\mathbf{M}^{-1}\mathbf{M}_m)\mathbf{v}_m\right] \tag{28}$$

It is now useful to distinguish between two contributions in the momentum equations for cells and ECM:

- Contributions due to the interactions with the extracellular liquid and to the pressure gradient;
- Contributions related to the interaction between cells and between cells and ECM.

In many cases the former are less important and can be dropped. Then the momentum equations can be simplified into

$$\begin{aligned} &\nabla\cdot(\phi_i\mathbf{T}_{\phi_i}) + \sum_{\substack{j=1\\ j\neq i}}^{I}\mathbf{m}_{ij} + \mathbf{m}_{im} = \mathbf{0}, \qquad i = 1,\ldots,I, \\ &\nabla\cdot(m\mathbf{T}_m) - \sum_{i=1}^{I}\mathbf{m}_{im} = \mathbf{0}. \end{aligned} \tag{29}$$

Under these hypotheses Eq. (29) does not depend on the interstitial pressure or on the liquid velocity. Therefore, they can in principle be solved without solving (27) and (28). Integration of (27) and (28) is only required if we want to describe the evolution of either the interstitial pressure, or of the liquid velocity. They are obtained in cascade after integrating the equations (29) above.

Regarding the behaviour of the cell population, we can assume that they respond to the compression of other cells independently from their type, i.e.

$$\mathbf{T}_{\phi_i} = \mathbf{T}_\phi. \tag{30}$$

An additional requirement is that the sum of the equations for the cell constituents yields

$$\nabla \cdot (\phi \mathbf{T}_\phi) + \sum_{i=1}^{I} \mathbf{m}_{im} = \mathbf{0} \,. \tag{31}$$

This can be achieved assuming that the cells belonging to the i-th population press those of the j-th population with a force proportional to $\nabla \cdot (\phi_i \mathbf{T}_\phi)$, and at the same time are pressed by the latter with a force proportional to $\nabla \cdot (\phi_j \mathbf{T}_\phi)$. In view of an integral balance law, these contributions need to be multiplied by the volume ratio of the population they act upon, with reference to the overall cellular component of the mixture ϕ. This suggests that the net interaction force $\mathbf{m}_{ij}$ might take the following form

$$\mathbf{m}_{ij} = \frac{\phi_i}{\phi} \nabla \cdot (\phi_j \mathbf{T}_\phi) - \frac{\phi_j}{\phi} \nabla \cdot (\phi_i \mathbf{T}_\phi), \tag{32}$$

so that the momentum equations for the cell populations specialize $(29)_1$ as

$$\frac{\phi_i}{\phi} \nabla \cdot (\phi \mathbf{T}_\phi) + \mathbf{m}_{im} = \mathbf{0} \,, \qquad i = 1, \ldots, I \,, \tag{33}$$

that sum up to the force balance equation for the sub-mixture of cells (31).

We observe that summing all Eqs.(33) and $(29)_2$ gives the force balance equation for the tissue

$$\nabla \cdot (\phi \mathbf{T}_\phi + m \mathbf{T}_m) = \mathbf{0} \,, \tag{34}$$

with the pressure gradient term and the interaction forces with the liquid neglected, compatibly with the assumptions done before writing Eqs.(29).

Let us focus now more specifically on the interaction between cells and ECM which of course depends on both the volume ratios of the ECM constituents and of the cells, and therefore also on the available portion of space ℓ occupied by the liquid.

Though as a first approximation one can still assume the interaction terms to be proportional to the velocity differences as done when the liquid phase was involved, i.e.,

$$\mathbf{m}_{im} = -\mathbf{M}_{im} (\mathbf{v}_i - \mathbf{v}_m) \,, \tag{35}$$

a better description of the attachment/detachment mechanisms between cells and ECM would be desirable.

An alternative form for the interaction terms is proposed in [44] on the basis of the experiments performed by Baumgardner et al. [7], Canetta et al. [14], and Sun et al. [51] who measured the adhesive strength of a cell attached to a microsphere linked to the tip of an atomic force microscopy cantilever. The microsphere might present proper adhesion molecules on its surface in

order to check the specific interaction of the cell adhesion molecule with those on the tip of the cantilever.

After putting the microsphere in contact with the cell, the cantilever is pulled away at a constant speed (in the range 0.2–4 μm/sec). If there is no adhesion between the microsphere and the cell, the force measured presents no stretching when the microsphere is taken away from the cell. This is experimentally obtained, for instance, by the addition of an antibody attaching to the external domain of the adhesion molecule, or by interfering with the links between the adhesion molecules and the cell cytoskeleton.

On the other hand, adhesion gives rise to the measurement of a stretching force and a characteristic jump indicating the rupture of an adhesive bond. Therefore these bonds have a limited strength quantified to be in the range 35–55 pN each by Baumgardner et al. [7].

A similar result was also obtained by Sun et al. [51] who did not functionalize the microsphere and allowed a longer resting period on the cell surface, ranging from 2 to 30 seconds. Again, pulling away the cantilever at a constant speed in the range 3–5 μm/sec caused the rupture of one or more adhesive bonds. They used different cell types (Chinese hamster ovary cells, endothelial cells and human brain tumour cells), all showing an adhesive strength of a single bond slightly below 30 pN. Coating the bead with poly-L-lysine or collagen did not lead to significant changes in the measurement.

On the other hand, big differences were observed interfering with the adhesion mechanism either by capping the external domain of the adhesion molecule with a proper antibody [7], or by disrupting the actin cytoskeleton [51], or by eliminating the link between adhesion molecules and the cytoskeleton [14].

It is not trivial to quantitatively transfer this measurement done at a molecular scale to a constitutive law at a tissue scale. However, we can say that this phenomenological description suggests that if cells are not pulled strong enough to detach from the ECM, they remain attached to it. If they detach the force in excess can be assumed to be proportional to the velocity difference, as suggested by viscoplasticity theory.

This translates into the following constitutive assumption

$$
\begin{aligned}
&\mathbf{v}_i = \mathbf{v}_m\,, \qquad \text{if} \quad |\mathbf{m}_{im}| \le \sigma_{im}\,,\\
&(|\mathbf{m}_{im}| - \sigma_{im})\frac{\mathbf{m}_{im}}{|\mathbf{m}_{im}|} = \alpha_{im}(\mathbf{v}_m - \mathbf{v}_i)\,, \qquad \text{if} \quad |\mathbf{m}_{im}| > \sigma_{im}\,.
\end{aligned}
\tag{36}
$$

The coefficient σ_{im} can be compared to a friction force and as such it is expected to depend on the adhesion mechanisms and on the volume ratio of the actors, the cells and the ECM. Proportionality between velocity and the force is a starting assumption made in absence of more precise experimental data.

From (33)

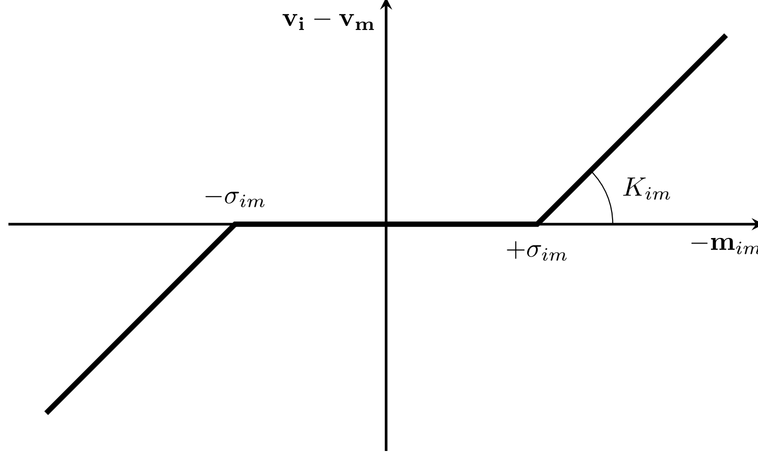


Fig. 2. Viscoplastic cell–ECM interaction.

$$\mathbf{m}_{im} = -\frac{\phi_i}{\phi} \nabla \cdot (\phi \mathbf{T}_\phi) \,,$$

and therefore Eq. (36) can be rewritten as

$$\mathbf{v}_i - \mathbf{v}_m = K_{im} \left(\frac{\phi_i}{\phi} - \frac{\sigma_{im}}{|\nabla \cdot (\phi \mathbf{T}_\phi)|} \right)_+ \nabla \cdot (\phi \mathbf{T}_\phi) \tag{37}$$

where $(\cdot)_+$ stands for the positive part of the parenthesis and $K_{im} = \alpha_{im}^{-1}$ is called in this chapter motility coefficient of the i-th cell population. This behaviour is presented in Figure 2. Equation (37) replaces Eq. (33) and has to be solved jointly with Eq. $(29)_2$ or (34).

To better understand the meaning of the constitutive equations above we can make some calculations for $|\mathbf{m}_{im}| > \sigma_{im}$. Taking the modulus of Equation (36), one has

$$\alpha_{im} |\mathbf{v}_m - \mathbf{v}_i| = |\mathbf{m}_{im}| - \sigma_{im} \,.$$

Replacing $|\mathbf{m}_{im}|$ in the same equation, it can be rewritten as

$$\alpha_{im} |\mathbf{v}_i - \mathbf{v}_m| \frac{\mathbf{m}_{im}}{\sigma_{im} + \alpha_{im} |\mathbf{v}_i - \mathbf{v}_m|} = \alpha_{im} (\mathbf{v}_m - \mathbf{v}_i) \,,$$

or

$$\mathbf{m}_{im} = \sigma_{im} \frac{\mathbf{v}_m - \mathbf{v}_i}{|\mathbf{v}_m - \mathbf{v}_i|} + \alpha_{im} (\mathbf{v}_m - \mathbf{v}_i) \,,$$

which allows to distinguish in $\mathbf{m}_{im}$ a static contribution in the direction of the relative motion (the first term) from a drag contribution proportional to the velocity difference. Of course, a viscous drag force is recovered in the limit

$\sigma_{im} = 0$. As already mentioned the friction force σ_{im} strongly depends on the concentration of ECM. For instance, increasing the concentration of ECM leads to an increase in activated adhesion sites and therefore in a stronger friction threshold. In addition, it is known that there is an optimal concentration of ECM favouring motility, because the content of ECM can not become too small, otherwise the lack of substratum would lead to a decrease in cell motility. Then the observation that cells hardly move when there is little or too much ECM can be translated as σ_{im} increasing for small and "large" m, thus, effectively, prohibiting cellular motion.

5 Stress Tensors

The momentum equations discussed in the previous sections need to be accompanied by the constitutive equations describing the response of the cells and ECM component to stress.

The basic questions are: How does the tumour behave? Is it a liquid or a solid? How should we summarize in a macroscopic constitutive equation cell adhesion properties? Should we take viscous or viscoelastic effects into account? What about plastic or viscoplastic deformations? Does a multicellular spheroid possess surface tension?

These questions are not at all trivial and there is no definitive answer yet, especially because of the lack of experiments. In fact, from the experimental point of view it is very difficult to perform mechanical tests on living matter, and in particular on ensembles of cells. In this respect Winters et al. [52] performed a wonderful and very promising experiment consisting in a uni-axial compression test. More precisely, multicellular spheroids with radii in the range 0.15–0.7 mm were positioned between two plates immersed in a physiological liquid. The lower plate was raised and the force acting on the upper plate was measured using a Cahn electrobalance. Two different compressions were performed for each multicellular spheroid in order to check whether an elastic model or a liquid model with surface tension was more proper to describe the mechanical response. In the latter case the measured surface tension should be independent of the deformation, in the former case it would not and their ratio should be close to ratio of measured force. Of course, as stated in the paper, only measurements in which surface tension is independent of the applied force and size can be used to calculate for each cell line the value of surface tension. In most cases they concluded that the multicellular spheroid behaves like a liquid. However, in other cases they found that the behaviour was elastic (see Table 1). They argue that this might be explained with a production of ECM by the cells in the multicellular spheroid.

Unfortunately, uniaxial test can not exclude that what they are measuring is actually the yield stress that need to be imposed before rupturing the adhesion sites among molecules. It would be interesting to perform shear

Table 1. Force ratio and deduced surface tension measurement for different cell lines (data from [52]).

Cell line	Surface tension (dynes/cm±SEM)	ratio	force ratio	material
U-87MG	6.9 ± 0.4 and 7.1 ± 0.3	1.0	1.6	liquid
U-118MG	16.3 ± 0.3 and 17.2 ± 0.5	1.1	1.5	liquid
LN-229 TE/C	10.3 ± 0.3 and 10.0 ± 0.4	1.0	1.5	liquid
LN-229 TE	8.2 ± 0.9 and 12.4 ± 0.9	1.6	1.6	elastic

tests. This would establish with no doubt at what class of material we should look at to obtain a good constitutive equation to describe tumour growth.

Some results in this direction are obtained by Iordan and Verdier (to be published). They put in a plate-and-plate rheometer a cell suspension at different concentrations proving the existence of a yield stress at higher concentrations (say, greater than 40%, i.e., $\phi > 0.4$).

Another theoretical difficulty comes when one wants to describe tumour as solids, because they are growing, remodelling and re-organising while deforming. This brings to two difficulties that need to be properly addressed if we want to describe tumours as solid masses

- defining a reference configuration with respect to what we can measure deformations;
- defining a proper Lagrangian coordinate system.

More precisely, following the ideas presented in [25, 46] (see also [33]), one needs to describe how the natural configuration evolves in time due to growth and internal re-organisation. Ambrosi and Mollica [1, 2] use a purely elastic one–component model to evaluate residual stress formation in a growing multicellular spheroid. This approach was developed in [4] working in a multiphase framework and taking also internal re-organisation and ECM deformation into account. This gave rise to an elasto-viscoplastic description for the cell population and a compressive elastic description for the ECM.

We refrain from entering in detail in this type of constitutive models, because it is too lengthy to fit this chapter. We suggest our reader to refer to the works mentioned above for further details on this topic.

We also refrain from dealing with viscoelastic constitutive models for a different reason. In fact, though viscoelastic characteristics are important in describing the mechanical behaviour of tissues, they are less important in describing growth. This is due to the fact that the characteristic times of the mechanical response of biological materials are of the order of tens of seconds (see for instance, Forgacs et al. [17]), and therefore much less than the characteristic times of cell duplication (a day). Therefore, the effect of viscoelasticity fades away quite quickly with respect to the time the material requires to grow leaving only a viscous heritage [43].

In most of the multiphase models of tumour growth deduced in the literature the tumour is modelled as a fluid. This approach, in fact, circumvents the difficulties mentioned above, mainly for two related reasons:

- the stress depends on the volume ratios and on the rate of deformations;
- it is possible to use an Eulerian approach.

Of course, tissues are not liquid and as stated above even ensembles of cells are unlikely to behave as a liquid. However, in this modelling approach the "cellular liquid" is contained in a solid structure of the ECM, so the material as a whole would look like a viscoelastic solid.

The easiest constitutive equation for the ensemble of cells consists in assuming that they behave as an elastic fluid, i.e.,

$$\mathbf{T}_\phi = -\Sigma \mathbf{I}\,,$$

where Σ is taken positive in compression. The use of this constitutive equation would result in a multicellular spheroid that in absence of ECM is not able to sustain shear.

In this case one can substitute (37) in the mass balance equations to obtain the following model

$$\begin{cases} \dfrac{\partial \phi_i}{\partial t} + \nabla\cdot(\phi_i \mathbf{v}_m) + \nabla\cdot\left[\phi_i K_{im}\left(\dfrac{\phi_i}{\phi} - \dfrac{\sigma_{im}}{|\nabla(\phi\Sigma)|}\right)_+ \nabla(\phi\Sigma)\right] + \mathit{\Gamma}_{\phi_i}\,, \\ \dfrac{\partial m_j}{\partial t} + \nabla\cdot(m_j \mathbf{v}_m) = \mathit{\Gamma}_{m_j}\,, \\ \nabla\cdot(m\mathbf{T}_m) - \nabla(\phi\Sigma) = \mathbf{0}\,, \end{cases} \tag{38}$$

for $i = 1, \ldots, I$ and $j = 1, \ldots, J$.

A possible extension is to consider a viscous behaviour as done in [13, 18, 19, 20]

$$\hat{\mathbf{T}}_T = (-\Sigma + \lambda\nabla\cdot\mathbf{v}_\phi)\mathbf{I} + 2\mu\mathbf{D}\,, \tag{39}$$

where $\mathbf{D} = (\nabla\mathbf{v}_\phi + \nabla\mathbf{v}_\phi^T)/2$ is the rate of strain tensor. This constitutive equation has the advantage to confer more stability to the growing mass.

6 Tumour Growth in a Rigid ECM

Coming to applications we start with the easiest case, when the ECM is rigid and does not change. The reader has to be aware of the fact that an immediate consequence of this hypothesis would be that from the macroscopic point of view the tissue would behave like a rigid porous medium, with cells and water moving inside a rigid scaffold. Any stress acting on the bulk tissue would be sustained by the ECM and cells in the core of the tissue would experience no stress deriving directly from the external actions.

From the mathematical point of view, this means that the stress tensor $\mathbf{T}_m$ acts as a (tensor) Lagrangian multiplier to satisfy the constraint $\mathbf{v}_m = \mathbf{0}$.

As an example, we apply the modelling approach above to the growth of a tumour in a host enviroment using only two cell populations, tumour cells with volume ratio ϕ_t and host cells with volume ratio ϕ_n initially occupying different domains $\Omega_t(t=0)$ and $\Omega \setminus \Omega_t(t=0)$.

The interface $\partial\Omega_t(t)$ between tumour and environment is a material surface moving with the common velocity of the cells

$$\mathbf{n} \cdot \frac{d\mathbf{x}_t}{dt} = \mathbf{n} \cdot \mathbf{v}_t = \mathbf{n} \cdot \mathbf{v}_n \,, \qquad \text{on} \quad \partial\Omega_t(t) \,. \tag{40}$$

It can be shown that the two cell populations stay segregated at all times. Taking into account that the interface conditions are enforced through continuity of stress and velocity, and treating for sake of simplicity the ensemble of cells as elastic fluids, we have the following free boundary problem:

$$\begin{cases} \dfrac{\partial\phi_t}{\partial t} + \nabla \cdot (\phi_t \mathbf{v}_{\phi_t}) = \Gamma_{\phi_t} \,, \qquad \text{in } \Omega_t \,, \\ \mathbf{v}_t = -K_{tm} \left(1 - \dfrac{\sigma_{tm}}{|\nabla \cdot (\phi_t \Sigma)|}\right)_+ \nabla(\phi_t \Sigma) \,, \qquad \text{in } \Omega_t \,, \\ \dfrac{\partial\phi_n}{\partial t} + \nabla \cdot (\phi_n \mathbf{v}_{\phi_n}) = \Gamma_{\phi_n} \,, \qquad \text{in } \Omega - \Omega_t \,, \\ \mathbf{v}_n = -K_{nm} \left(1 - \dfrac{\sigma_{nm}}{|\nabla \cdot (\phi_n \Sigma)|}\right)_+ \nabla(\phi_n \Sigma) \,, \qquad \text{in } \Omega - \Omega_t \,, \\ \mathbf{v}_t \cdot \mathbf{n} = \mathbf{v}_n \cdot \mathbf{n} \,, \qquad \text{on } \partial\Omega_t \,, \\ \phi_t \Sigma(\phi_t) = \phi_n \Sigma(\phi_n) \,, \qquad \text{on } \partial\Omega_t \,. \end{cases} \tag{41}$$

Since the cell populations stay segregated, it is possible to introduce only one variable for the volume ratio of cells: ϕ, and assume that ϕ is the volume ratio of tumour cells ϕ_t in Ω_t and the volume ratio of normal host cells ϕ_n in Ω_h:

$$\phi = \begin{cases} \phi_t, & \text{in } \Omega_t \,, \\ \phi_n, & \text{in } \Omega \setminus \Omega_t \,. \end{cases}$$

We can rewrite the main equation as

$$\frac{\partial\phi}{\partial t} = \nabla \cdot \left[\phi K_m \left(1 - \frac{\sigma_m}{|\nabla \cdot (\phi\Sigma)|}\right)_+ \nabla(\phi\Sigma) \right] + \Gamma \,, \tag{42}$$

where, for instance, $K_m = K_{tm}$ in Ω_t and $K_m = K_{nm}$ in $\Omega \setminus \Omega_t$. In this formulation it is natural to use level set methods with the interface dividing the domains moving according to (40).

In [16] $K_{tm} = K_{nm} = K_m$, $\sigma_{tm} = \sigma_{nm} = 0$, and $\Sigma = \Sigma(\psi)$ with $\psi = \phi_t + \phi_n + m$. In addition, the growth terms are modelled on the basis of the observation that when cells live in a crowded environment they sense the presence of other cells through the activation of mechano-transduction pathways. This phenomenon is called contact inhibition of growth and is one of the fundamental phenomena in controlling cell concentration. In the model this means that mitosis stops when the volume ratio (or the compression) overcomes a given threshold.

The behaviour of the cells in terms of growth and motion then crucially depends on how they feel the presence of other cells and how they translate the mechanical cues. What Chaplain et al. [16] showed is that if for instance, for some reason, e.g., a fault in the mechanotrasduction pathway, there is a misperception of the compression state of the local tissue and then of the subsequent stress which is exerted on a cell, then this degeneration can cause a clonal advantage on the surrounding cells leading to the replacement and the invasion of the healthy tissue with the formation of hyperplasia and therefore tumour lesions.

From the mathematical point of view the phenomenological description above can be formalized saying that the threshold value for tumour cells to overcome the restriction point and commit themselves to divide is slightly larger than the physiological one. Actually, it may even tend to infinity, meaning that the cells are completely insensitive to compression and continue replicating independently of the compression level.

We shall then consider the following growth terms

$$\Gamma_i = [\gamma_i H_\sigma(\psi - \psi_i) - \delta_i(\psi)]\phi_i \,, \qquad i = n, t \,, \tag{43}$$

where $\psi = \phi_n + \phi_t + m$. We assume that what makes the difference between a normal and a tumour cell stays in the growth term and in its dependence from the stress level.

Of course, cellular mechano-trasduction is not the only cause of formation of hyperplasia and tumours. In fact, chemical factors operate to regulate the reproduction rates so that the growth terms crucially depend on the presence of growth promoting factors, of growth inhibitory factors and of course of nutrients.

However, here we shall only focus on the possible role of stress on tumour invasion and therefore assume that all the constituents required to sustain growth and mitosis can be found abundant in the extracellular liquid.

In (43) $H_\sigma(\psi - \psi_i)$ is a mollifier of the step function, which is at least continuous, is constantly equal to 1 for ψ smaller than the threshold value ψ_i, and vanishes for $\psi > \psi_i + \sigma$. According to the discussion above the threshold values ψ_n and ψ_t are such that $\psi_n < \psi_t$. For the following discussion it is useful to observe that a balance between cell growth and death occurs when $\gamma_i H_\sigma(\psi - \psi_i) = \delta_i(\psi)$, or, in the case in which δ_i is considered constant as in the following simulations

$$\psi = \psi_i + H_\sigma^{-1}\left(\frac{\delta_i}{\gamma_i}\right) , \tag{44}$$

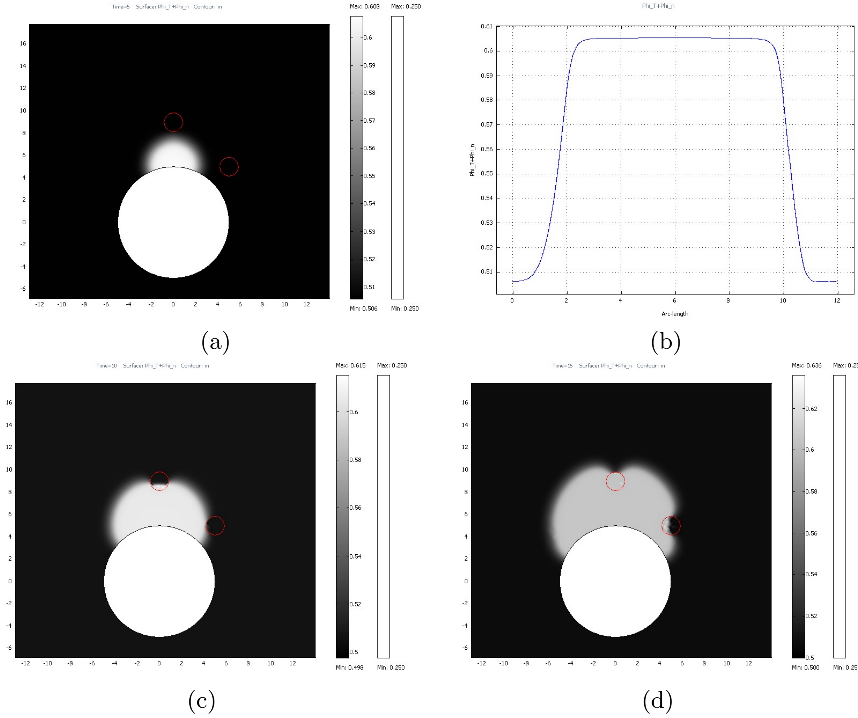


Fig. 3. Growth of a tumour in a heterogeneous tissue surrounding a bone. Denser ECM is found near the points (0,10) and (5,5). (a), (c), and (d) give the cell volume ratio $\phi_t + \phi_n$ at the dimensionless times 5, 10, and 15. (b) gives the cell volume at the section of the tumour along $y = 6$ for $x \in [-6, 6]$ and $t = 5$.

The simulation in Figure 3 shows how some tumour cells originating from the surface of a bone diffuse in the surrounding tissue. Initially, the interface dividing the tumour from the host tissue is nearly circular. Far away from the tumour mass the volume ratio occupied by the cells is that given in (44) with $\psi_n = 0.5$, while in the core of the tumour it is that corresponding to $\psi_n = 0.6$. Near the surface it is possible to observe a compression of the host tissue as put in evidence by a cross section ($y = 0.6$) in Figure 3b, showing the cell volume ratio. The interface between the two is located near the level $\phi_t + \phi_n = 0.59$. However, growth is not occurring in a homogeneous tissue. In fact, while in most of the domain the volume ratio occupied by the ECM is 0.2, there are two regions centered in (0,10) and (5,5) with a higher volume ratio increasing up to 0.3. The presence of these heterogeneities breaks the

symmetry of the tumour, as already evident in Figure 3b. Figures 3c,d show how the presence of more ECM slows down tumour invasion

7 Tumour Growth in a Remodelling ECM

With little effort we can adjust the model to take ECM remodelling into account. This is essential to describe the formation of fibrotic tissues and therefore of stiffer stroma.

So we to additionally consider

- the volume ratio m occupied by the extracellular matrix;
- the concentration c of matrix degrading enzymes (MDEs).

The numerous constituents of the extracellular matrix, are produced in a stress-dependent way by the cells and are degraded by MDEs [15, 32, 41, 50].

Hence the remodelling process can be described by adding an equation for m:

$$\frac{\partial m}{\partial t} = \mu_n(\Sigma)\phi_n + \mu_t(\Sigma)\phi_t - \nu c m \,, \tag{45}$$

where μ_n and μ_t are the ECM production rates respectively by normal and tumour cells and ν is the degradation coefficient due to the action of MDEs.

Active MDEs are produced (or activated) by the cells, diffuse throughout the tissue and undergo some decay (either passive or active). So one has to introduce the following reaction-diffusion equation governing the evolution of MDE concentration

$$\frac{\partial c}{\partial t} = \kappa \nabla^2 c + \pi_n(\Sigma)\phi_n + \pi_t(\Sigma)\phi_t - \frac{c}{\tau} \,. \tag{46}$$

where π_n and π_t are the MDE production rates respectively by normal and tumour cells and τ is its half life.

In (45) it is important that the production coefficients of ECM by normal and tumour cells be different in order to describe the formation of fibrosis characterizing many tumours. Also the functions π_n and π_t describe the production levels of active MDEs by normal and tumour cells, respectively. They may be different and certainly depend on the compression level. As proved in [16] they also can be the cause of the formation of fibrotic tissues.

Thus the complete system of equations with matrix remodelling effects taken into account is:

$$\begin{cases} \dfrac{\partial \phi_n}{\partial t} = \nabla \cdot [\phi_n K_m \nabla(\phi_n \Sigma(\psi))] + \gamma_n H_\sigma(\psi - \psi_n)\phi_n - \delta_n(\psi)\phi_n \,, \\ \dfrac{\partial \phi_t}{\partial t} = \nabla \cdot [\phi_t K_m \nabla(\phi_t \Sigma(\psi))] + \gamma_t H_\sigma(\psi - \psi_t)\phi_t - \delta_t(\psi)\phi_t \,, \\ \dfrac{\partial m}{\partial t} = \mu_n(\Sigma)\phi_n + \mu_t(\Sigma)\phi_t - \nu c m \,, \\ \dfrac{\partial c}{\partial t} = \kappa \nabla^2 c + \pi_n(\Sigma)\phi_n + \pi_t(\Sigma)\phi_t - \dfrac{c}{\tau} \,. \end{cases} \tag{47}$$

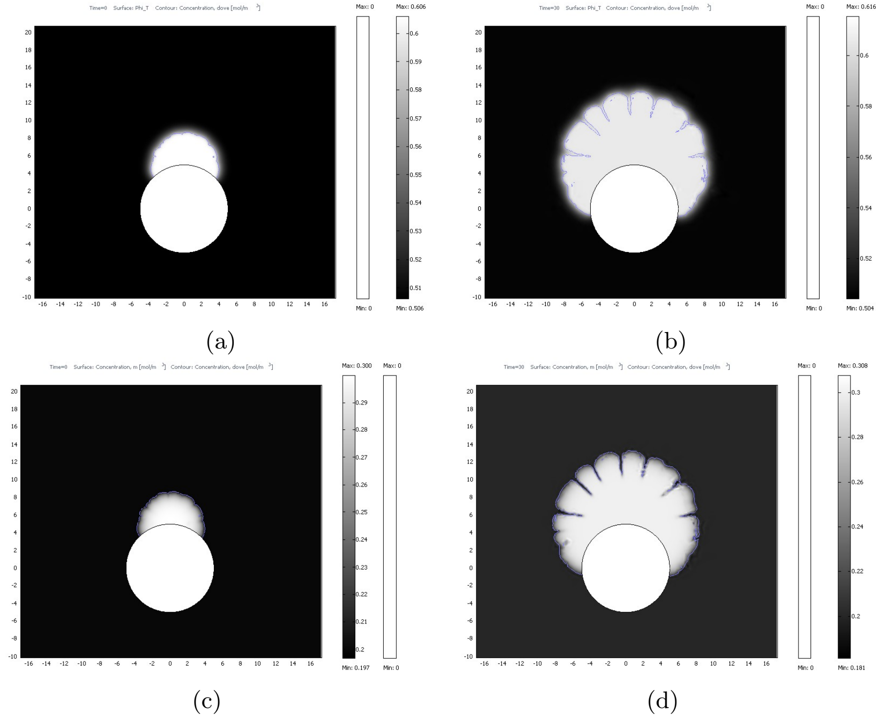


Fig. 4. Growth of the fibrosis in a homogeneous tissue surrounding a bone. Cell volume ratio $\phi_t + \phi_n$ at the dimensionless times 30 and 60 is given in (a) and (b). ECM volume ratio is given in (c) and (d). The line delimits the tumour from the host tissue.

One of the by-product of this model is the description of the formation of fibrotic tissues and of tissues stiffer than normal so that they may be sometimes felt with a self-test. This is the aim of the simulation shown in Figure 4. The situation is similar to the previous one. However, now the ECM is initially distributed homogeneously with $m = 0.2$. On the other hand, while prolifer-

ating tumour cells will produce matrix degrading enzyme as the host cells but will produce more extracellular matrix than normal. This brings the formation of a tumour characterized by an amount of ECM with a volume ratio close to $m = 0.3$. From the mechanical point of view this increase in the percentage of ECM would lead to an increase of almost one order of magnitude in tissue stiffness.

8 Vascularized Tumour Growth

The general model can be modified to describe vascular tumour growth. To start consider the most simple configuration: a vessel and tumour tissue in the direct vicinity of the vessel. As a vessel is a natural source of oxygen and nutrients the region near the vessel is beneficial for tumour growth. On the contrary, regions very distant from the vessel are likely to be prohibitive for the tumour.

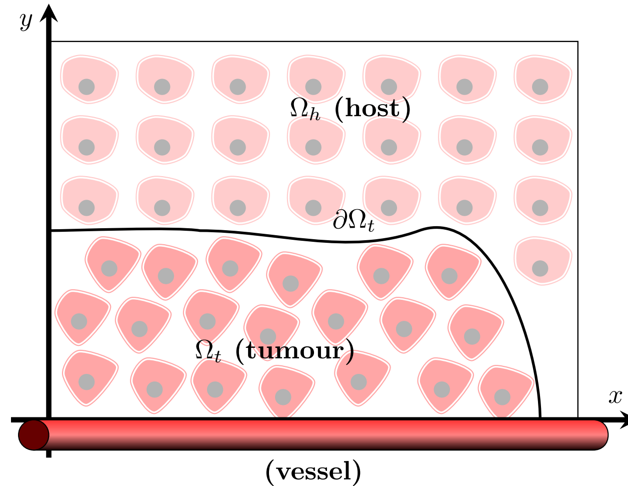


Fig. 5. Tumour cord region Ω_t and region of host tissue Ω_h. Blood vessel is positioned along the x-axis ($\partial\Omega_{south}$).

This configuration may lead to the formation of a tumour along the vessel that we shall call *tumour cord*.

Let us consider a two-dimensional domain Ω where tumour occupies the region Ω_t, and the rest of the domain $\Omega_h = \Omega \setminus \Omega_t$ is occupied by the normal host tissue (Fig. 5).

We assume that there is a blood vessel which coincides with some of the boundaries. In particular we shall consider the case when there is a vessel along the x-axis.

The basic model is given in Eq.(41). However, for sake of simplicity, we shall neglect any viscoplastic effects, effectively substituting $\sigma_{im} = 0$, $i = t, n$.

The model should be accompanied by the selection of an appropriate growth term. One might expect that any growth process within the tissue is closely related to cell metabolism and energy balance. Thus we need to introduce equations governing the distribution and consumption of various nutrients, like Eq. (15).

The type of the tumour metabolism assumed shall define which nutrients are of the most interest and should be included in the model.

For example, let us consider glucose catabolism. In normal conditions its fission produces approximately 32 molecules of ATP per molecule of glucose [38] and requires 6 molecules of oxygen. However incomplete glucose oxydation is also possible in hypoxic condition. In this case there are only 2 molecules of ATP produces per molecule of glucose, and the by-product in this case is lactic acid (Fig. 6).

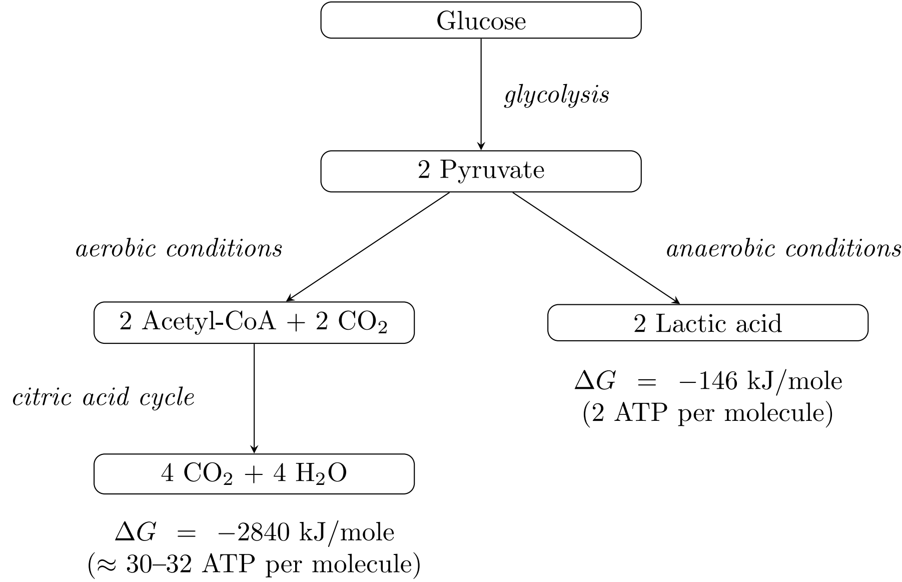


Fig. 6. Glucose catabolic pathways (based on [38]). Anaerobic pathway is less energy efficient and in addition it acidifies the microenvironement, but many tumours rely on this type of metabolism.

While anaerobic pathway is less energy efficient, it proved to give an evolutionary advantage to tumours as they tend to develop and heavily rely on their ability for glycolysis oriented metabolism [48, 22, 21].

Thus the proper description of the metabolism is essential for obtaining a model, valid for a wide range of tumours. In the most simple case one may assume that the tumour has not yet developed an ability for anaerobic metabolism. In this case one may show that it is only concentration of oxygen that is important within the model.

Let c be the concentration of oxygen, and its distribution be governed by reaction–diffusion equation

$$\frac{\partial c}{\partial t} = D\Delta c + \mathrm{G}(c, \phi),$$

where G is the oxygen consumption term:

$$\mathrm{G}(c, \phi) = \begin{cases} \alpha\phi \mathrm{f}(\phi)c, & \text{in } \Omega_t \\ 0, & \text{in } \Omega_h, \end{cases}$$

We assume that consumption of the host tissue is negligible with respect to growing tumour tissue. Rate of oxygen uptake is described by α. Function $\mathrm{f}(\phi)$ characterizes intensity of metabolic processes: we expect that cells stop proliferating (and consuming) at maximum packing density ϕ_*.

Then we may define a growth/death term of the cellular phase in the tumour subdomain as

$$\Gamma = \gamma\phi(\mathrm{f}(\phi)c - \theta)_+ - \epsilon\phi(\theta - \mathrm{f}(\phi)c)_+,$$

where γ and ϵ are growth and death rates respectively, θ is minimal life maintenance cost per cell, and $(\cdot)_+$ denotes the positive part of $(\cdot)$. More details on how this form of the growth term is obtained can be found in [6].

For the host subdomain Ω_h we assume

$$\Gamma \equiv 0.$$

One may refer to [6] for further details on this kind of models.

Let both tissues have identical mechanical properties, and have the same stress-free packing density ϕ_0, then Σ may be chosen as

$$\Sigma(\phi) = \phi - \phi_0.$$

Initially both tissues are in stress free condition:

$$\phi(x, y, 0) = \phi_0 \tag{48}$$

We distinguish three kinds of exterior boundaries:

- a boundary coinciding with a blood vessel,
- a remote boundary,
- a symmetry axis.

For the vascular boundary we assume that the cells do not penetrate into the vessel, and oxygen supply is always sufficient to maintain its constant concentration c_{in}:

$$\frac{\partial \phi \Sigma(\phi)}{\partial \mathbf{n}}(x,y,t) = 0, \quad c(x,y,t) = c_{in}, \quad \text{for } (x,y) \text{ at vascular boundaries,} \tag{49}$$

where $\mathbf{n}$ is an external normal of Ω.

For remote boundaries we assume that they stay undisturbed by the growth and there is zero flux of oxygen through those boundaries:

$$\phi(x,y,t) = \phi_0, \quad \frac{\partial c}{\partial \mathbf{n}}(x,y,t) = 0, \quad \text{for } (x,y) \text{ at remote boundaries.} \tag{50}$$

And for a symmetry axis we use

$$\frac{\partial \phi \Sigma(\phi)}{\partial \mathbf{n}}(x,y,t) = 0, \quad \frac{\partial c}{\partial \mathbf{n}}(x,y,t) = 0, \quad \text{for } (x,y) \text{ at an axis of symmetry.} \tag{51}$$

In the configuration shown in Fig. 5 there are five boundaries: the four exterior boundaries of model domain Ω, and $\partial\Omega_{th}$, the boundary between Ω_t and Ω_h, or tumour–host interface.

We consider the right and the top boundaries to be remote, the bottom boundary to coincide with the vessel, and the left boundary to be a symmetry axis.

For the tumour–host interface $\partial\Omega_{th}$ we assume continuity of stress (this implies continuity of ϕ). For mass preservation reasons the velocity of the interface should be

$$\mathbf{v}_{\partial\Omega_{th}} \cdot \mathbf{n} = -K_m \nabla(\phi\Sigma(\phi)) \cdot \mathbf{n},$$

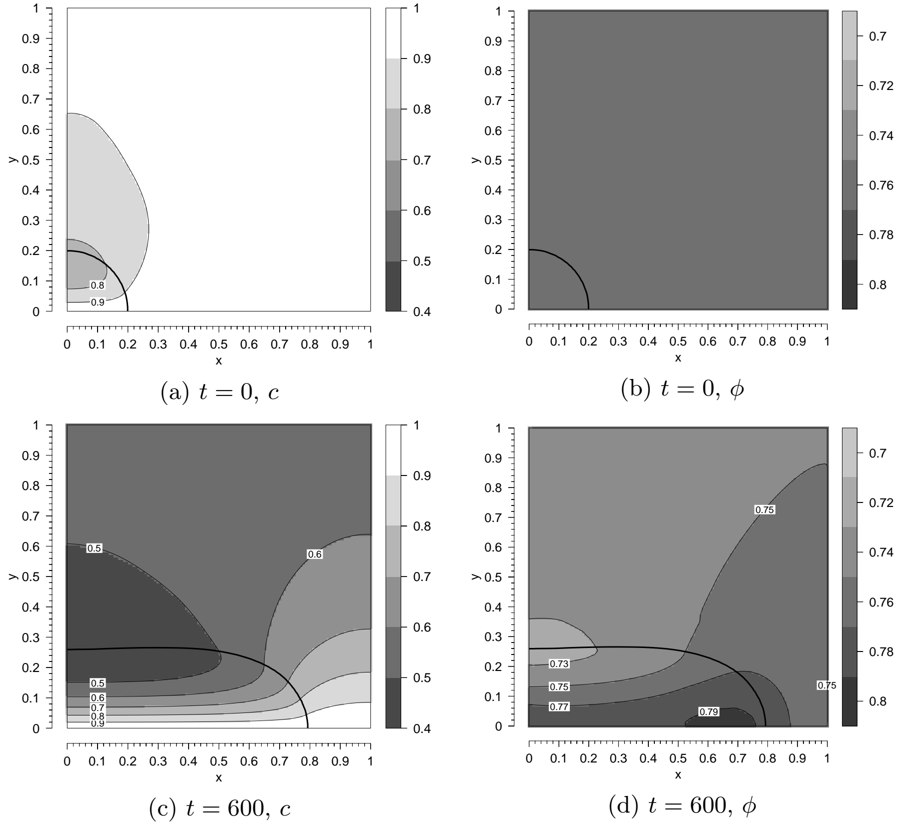
where $K_m = K_{tm} = K_{nm}$.

A typical cord growth in single-vessel configuration is shown at Fig. 7. Initial cord size was 0.2, and the values of the other parameters are $\gamma = 1$, $\epsilon = 0.8$, $\alpha = 200$, $K_m = 0.01$, $\theta = 0.15$. Stress free density was $\phi_0 = 0.75$, $f(\phi) = 1 - \phi$, $D = 1$, $c_{in} = 1$.

The source code used for computer simulations using the model is freely available at `http://code.google.com/p/cord/`.

One may observe that the growth of the tumour region is highly anisotropic with the preferred direction of the vessel. The cord reaches and maintains the same radius along all the cord except the very tip. Packing density profile of the later stages suggests that it is at the tip of the cord where proliferation of the tumour is the most intensive. On opposite, in the region of the steady radius, there is a hypoxic region near the outer rim of the cord, and this is where cell death is most commonly observed in the simulation.

In general the behaviour of the tumour observed in the simulation confirms a well known observation that there exists a certain distance from the vessels above which the tumour may not go unless it develops ability to anaerobic metabolism.



(a) $t = 0$, c (b) $t = 0$, ϕ

(c) $t = 600$, c (d) $t = 600$, ϕ

Fig. 7. Cord growth. Thick line lines shows position of the tumour–host interface $\partial\Omega_{th}$. (a) and (c) show oxygen concentration. (b) and (d) show packing density profile.

9 Final Remarks and Open Problems

The general modelling framework illustrated in this chapter develops on the basis of the following observations.

- Tumour cells duplicate in a tissue characterized by the presence of other host cells, a deformable extra-cellular matrix made of many constituents and of extra-cellular liquid. If we want to model tumour growth from the macroscopic viewpoint, this induces the need to use a multiphase mathematical model with several constituents.
- Also due to the action of tumour cells themselves, the environment changes considerably during the evolution which in turn influences the behaviour of the cells.

- Cells are bound to the extracellular matrix through adhesion molecules, mainly integrins, that have a limited strength. In a similar way cells are also attached to other cells through other adhesion molecules, mainly cadherins, that also have a limited strength. On the basis of these experimental evidences it is proposed that there exists a threshold condition below which the ensemble of cells stick to the extracellular matrix and move with it. Above it cells gradually detach to move with respect to the extracellular matrix.

The model presented here can be specialized in several different ways, e.g., specifying the cells populations (endothelial cells, epithelial cells, fibroblasts, macrophages, lymphocytes), or including the different phases of the cell cycle, i.e. G_0, G_1, G_2, in view of the application of the model to the study of possible treatments, or distinguishing different tumour clones characterized by relevant differences in their behaviour, e.g., cells with normal and abnormal expression of the tumour suppressor gene, p53 and hormone sensitive and insensitive cells.

In this respect, one of the breakthrough in modelling tumour growth consists in including what happens inside the cells and therefore in developing multiscale models which take into account of the cascades of events regulating the behaviour of cells.

Another interesting problem which has not been studied yet is the growth of tumours in mechanically heterogeneous environment, which includes network structures like blood vasculature, airways, and limphatic system, the interaction with physical barriers like bones and cartilages, and the pressure on the surrounding tissues. This would allow to understand vessel collapse due to tumour growth, capsule formation and degradation, cell compartimentalization due to strong inhomogeneities of the ECM distribution, or tissue invasion related to changes in the adhesion mechanisms.

However, research in this direction still needs a characterization of the mechanical behaviour of growing tissues and their environment, in order to evaluate the importance of nonlinear effects, to quantify viscoelastic and plastic effect, and to identify the proper constitutive equation.

So, in developing all the generalizations above, one has to keep in mind the objective difficulties in obtaining specific measurements from experiments. For instance, characterizing from the mechanical viewpoint the behaviour of a growing and remodelling tissue is not easy. Quantifying the dependence of the production rates of extracellular matrix and matrix degrading enzymes from the level of stress and/or strain is not easy and data are not available yet, though the effects were put in evidence many years ago and related therapies applied in clinical practice, e.g., application of traction in bone healing and orthodoncy. For this reason, in our opinion, the development of mathematical models and of experiments have to run along parallel paths stimulating and cross-fertilizing each other.

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References

[1] Ambrosi, D., and Mollica, F., (2002). On the mechanics of a growing tumor, *Int. J. Engng. Sci.* **40**: 1297–1316.
[2] Ambrosi, D., and Mollica, F., (2004). The role of stress in the growth of a multicell spheroid, *J. Math. Biol.* **48**: 477–499.
[3] Ambrosi, D., and Preziosi, L., (2002). On the closure of mass balance models for tumor growth, *Math. Mod. Meth. Appl. Sci.* **12**: 737–754.
[4] Ambrosi, D., and Preziosi, L., (2007). Tumors as elasto-viscoplastic growing bodies. Submitted.
[5] Araujo, R.P., and McElwain, D.L.S., (2005). A mixture theory for the genesis of residual stresses in growing tissues, I: A general formulation, *SIAM J. Appl. Math.* **65**: 1261–1284.
[6] Astanin, S., and Tosin, A., (2007). Mathematical model of tumour cord growth along the source of nutrient, *Math. Mod. Nat. Phen.*, in press.
[7] Baumgartner, W., Hinterdorfer, P., Ness, W., Raab, A., Vestweber, D., Schindler, H., and Drenckhahn D., (2000). Cadherin interaction probed by atomic force microscopy, *Proc. Nat. Acad. Sci. USA* **97**: 4005–4010.
[8] Bear, J., and Bachmat, Y., (1990). **Introduction to Modeling of Transport Phenomena in Porous Media**, Kluwer Academic Publishers.
[9] Bowen, R.M., (1976) The theory of mixtures, in **Continuum Physics**, Vol. 3, Eringen, A.C., Ed., Academic Press.
[10] Breward, C.J.W., Byrne, H.M., and Lewis, C.E., (2002). The role of cell-cell interactions in a two-phase model for avascular tumor growth, *J. Math. Biol.* **45**: 125–152.
[11] Breward, C.J.W., Byrne, H.M., and Lewis, C.E., (2003). A multiphase model describing vascular tumor growth, *Bull. Math. Biol.* **65**: 609–640.
[12] Byrne, H.M., King, J.R., McElwain, D.L.S., and Preziosi, L., (2003). A two-phase model of solid tumor growth, *Appl. Math. Letters* **16**: 567–573.
[13] Byrne, H.M., and Preziosi, L., (2004). Modeling solid tumor growth using the theory of mixtures, *Math. Med. Biol.* **20**: 341–366.
[14] Canetta, E., Duperray, A., Leyrat, A., and Verdier, C., (2005). Measuring cell viscoelastic properties using a force-spectrometer: Influence of the protein-cytoplasm interactions, *Biorheology* **42**: 298–303.
[15] Chambers, A.F., and Matrisian, L.M., (1997). Changing views of the role of matrix metalloproteinases in metastasis, *J. Natl. Cancer Inst.* **89**: 1260–1270.
[16] Chaplain, M.A.J., Graziano, L., and Preziosi, L., (2006). Mathematical modelling of the loss of tissue compression responsiveness and its role in solid tumour development, *Math. Med. Biol.* **23**: 197–229.

[17] Forgacs, G., Foty, R.A., Shafrir, Y., and Steinberg, M.S., (1998). Viscoelastic properties of living embryonic tissues: a quantitative study, *Biophys. J.* **74**: 2227–2234.
[18] Franks, S.J., Byrne, H.M., King, J.R., Underwood, J.C.E., and Lewis, C.E., (2003). Modelling the early growth of ductal carcinoma in situ of the breast, *J. Math. Biol.* **47**: 424–452.
[19] Franks, S.J., Byrne, H.M., Mudhar, H.S., Underwood, J.C.E., and Lewis, C.E., (2003). Mathematical modelling of comedo ductal carcinoma in situ of the breast, *Math. Med. Biol.* **20**: 277–308.
[20] Franks, S.J., and King, J.R., (2003). Interactions between a uniformly proliferating tumor and its surrounding. Uniform material properties, *Math. Med. Biol.* **20**: 47–89.
[21] Gatenby, R.A., Gawlinski, E.T., Gmitro, A.F., Kaylor, B., and Gillies, R.J., (2006). Acid-meditated tumour invasion: a multidisciplinary study, *Cancer Res.* **66**: 5216–5223.
[22] Gillies, R.J., and Gatenby, R.A., (2007). Hypoxia and adaptive landscapes in the evolution of carcinogenesis, *Cancer Metastasis Rev.*, E-publication
[23] Graziano, L., and Preziosi, L., (2007). Mechanics in tumour growth, in **Modeling of Biological Materials**, Mollica, F., Preziosi, L., and Rajagopal, K.R., Eds., Birkhäuser, 267–328.
[24] Hou, J.S., Holmes, M.H., Lai, W.M., and Mow, V.C., (1989). Boundary conditions at the cartilage-synovial fluid interface for joint lubrication and theoretical verifications, *J. Biomech. Engng.* **111**: 78–87.
[25] Humphrey, J.D., and Rajagopal, K.R., (2002). A constrained mixture model for growth and remodeling of soft tissues, *Math. Mod. Meth. Appl. Sci.* **12**: 407–430.
[26] Jayaraman, G., (1983). Water transport in the arterial wall. A theoretical study, *J. Biomech.* **16**: 833–840.
[27] Kenion, D.E., (1979). A mathematical model of water flux through aortic tissue, *Bull. Math. Biol.* **41**: 79–90.
[28] Klanchar, M., and Tarbell, J.M., (1987). Modeling water flow through arterial tissue, *Bull. Math. Biol.* **49**, 651–669.
[29] Kwan, M.K., Lai, W.M., and Mow, V.C., (1990). A finite deformation theory of cartilage and other soft hydrated connective tissues: Part I – Equilibrium results, *J. Biomech.* **23**: 145–155.
[30] Lai, W.M., Hou, J.S., and Mow, V.C., (1990). A triphasic theory for the swelling properties of hydrated charged soft biological tissues, in **Biomechanics of Diarthroidal Joints**, Vol. **1**: 283–312, Springer.
[31] Lai, W.M., and Mow, V.C., (1980). Drag-induced compression of articular cartilage during a permeation experiment, *Biorheology* **17**: 111–123.
[32] Matrisian, L.M., (1992), The matrix–degrading metalloproteinases, *Bioessays* **14**: 455–463.
[33] Mollica, F., Preziosi, L., Rajagopal, K.R., (2007). **Modelling of Biological Materials**, Birkhäuser.
[34] Mow, V.C., Holmes, M.H., and Lai, W.M., (1984). Fluid transport and mechanical problems of articular cartilage: A review, *J. Biomech.* **17**: 377–394.
[35] Mow, V.C., Kuei, S.C., Lai, W.M., and Armstrong, C.G., (1980). Biphasic creep and stress relaxation of articular cartilage: Theory and experiment, *J. Biomech. Engng.* **102**: 73–84.
[36] Mow, V.C., and Lai, W.M., Mechanics of animal joints, (1979). *Ann. Rev. Fluid Mech.* **11**: 247–288.

[37] Mow V.C., et als., Eds., (1990). **Biomechanics of Diarthroidal Joints**, Springer.

[38] Nelson, D.L., and Cox, M.M., (2002). **I principi di biochimica di Lehninger**, Translated by M. Averna, E. Melloni, A. Sdraffa, Zanichelli.

[39] Nicholson, C., (1985). Diffusion from an injected volume of a substance in brain tissue with arbitrary volume fraction and tortuosity, *Brain Res.* **333**: 325–329,

[40] Oomens, C.W.J., Van Campen, D.H., and Grootenboer, H.J., (1987). A mixture approach to the mechanics of skin, *J. Biomech.* **20**: 877–885.

[41] Parson, S.L., Watson, S.A., Brown, P.D., Collins, H.M., and Steele, R.J.C., (1997). Matrix metalloproteinases, *Brit.J. Surg.* **84**: 160–166.

[42] Preziosi, L., and Farina, A., (2001). On Darcy's law for growing porous media, *Int. J. Nonlinear Mech.* **37**: 485–491.

[43] Preziosi, L., and Joseph, D.D., (1987). Stokes' first problem for viscoelastic fluids, *J. Non-Newtonian Fluid Mech.* **25**: 239–259.

[44] Preziosi, L., and Tosin, A., (2007). Multiphase modeling of tumor growth and extracellular matrix interaction: Mathematical tools and applications, *J. Math. Biol.*, in press.

[45] Rajagopal, K.R., and Tao, L., (1995). **Mechanics of Mixtures**, World Scientific.

[46] Rao, I.J., Humphrey, J.D., and Rajagopal, K.R., (2003). Biological growth and remodeling: A uniaxial example with possible application to tendons and ligaments, *Comp. Mod Engr. Sci.* **4**: 439–455.

[47] Sarntinoranont, M., Chen, X., Zhao, J., Mareci, T.H., (2006). Computational model of interstitial transport in the spinal cord using diffusion tensor imaging, *Ann. Biomed. Eng.* **34**: 1304–1321.

[48] Smallbone, K., Gatenby, R.A., Gillies, R.J., Maini, P.K., and Gavaghan, D.J., (2007). Metabolic changes during carcinogenesis: Potential impact on invasiveness, *J. Theor. Biol.* **244**: 703–713.

[49] Sorek, S., and Sideman, S., (1986). A porous medium approach for modelling heart mechanics. Part 1: Theory, *Math. Biosci.* **81**: 1–14.

[50] Stetler-Stevenson, W.G., Hewitt, R., and Corcoran, M., (1996). Matrix metallo–proteinases and tumour invasion: From correlation to causality to the clinic, *Cancer Biol.* **7**: 147–154.

[51] Sun, M., Graham, J.S., Hegedus, B., Marga, F., Zhang, Y., Forgacs, G., and Grandbois, M., (2005). Multiple membrane tethers probed by atomic force microscopy, *Biophys. J.* **89**: 4320–4329.

[52] Winters, B.S., Shepard, S.R., and Foty, R.A., (2005). Biophysical measurement of brain tumor cohesion, *Int. J. Cancer* **114**: 371–379.

[53] Tsaturyan, A.K., Izacov, V.J., Zhelamsky, S.V., and Bykov, B.L., (1984). Extracellular fluid filtration as the reason for the viscoelastic behaviour of the passive myocardium, *J. Biomech.* **17**: 749–755.

[54] Yang, M., Taber, L.A., and Clark, E.B., (1994). A nonlinear poroelastic model for the trabecular embryonic heart, *J. Biomech. Engng.* **116**: 213–223.