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Mechano-transduction in tumour growth modelling*

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Abstract. The evolution of biological systems is strongly influenced by physical factors, such as applied forces, geometry or the stiffness of the micro-environment. Mechanical changes are particularly important in solid tumour development, as altered stromal-epithelial interactions can provoke a persistent increase in cytoskeletal tension, driving the gene expression of a malignant phenotype. In this work, we propose a novel multi-scale treatment of mechano-transduction in cancer growth. The avascular tumour is modelled as an expanding elastic spheroid, whilst growth may occur both as a volume increase and as a mass production within a cell rim. Considering the physical constraints of an outer healthy tissue, we derive the thermo-dynamical requirements for coupling growth rate, solid stress and diffusing biomolecules inside a heterogeneous tumour. The theoretical predictions successfully reproduce the stress-dependent growth curves observed by *in vitro* experiments on multicellular spheroids.

1 Introduction

Recent advances in biosciences have highlighted that the evolution of biological systems is strongly influenced by physical factors, such as applied forces, geometry or the mechanical properties of the micro-environment [1]. Consequently, the study of mechano-transduction, that is the ensemble of processes converting mechanical forces into biochemically relevant factors, has become a multi-disciplinary subject attracting a growing research interest. Living materials typically encounter nano-scale to macroscopic forces which may change their nature from physiological to pathological conditions. Cells sense nano-scale forces by integrins and focal adhesion proteins, whose stimulus is converted into chemical activity by producing a transduction current that changes the membrane potential. In turn, actomyosin contractility gets activated for maintaining tensional homeostasis inside the tissue (*i.e.* the local balance between exogenous and endogenous forces), a process termed mechano-reciprocity [2]. This mechanism is fundamental during embryonic development, when the mechanical stiffness of the local environment is sensed by the developing cells, and transformed into contractile forces regulating the morphogenetic movements and cell differentiation [3]. Nevertheless, mechano-transduction is not limited to such switch-like events, but allows cells to respond to time-varying mechanical stimuli through dynamic molecular processes [4]. Therefore, cells undergo dynamic adaptation driven both by epigenetic remodelling and gene regulatory processes. In particular, gene expression may be triggered by long-term changes in micro-environment or cellular behaviour, causing a loss of tissue homeostasis which is often a hallmark of disease. Mechanical changes are particularly important for solid tumour development, as altered stromal-epithelial interactions often precede malignancy. In particular, the healthy extra-cellular matrix is changed by remodelling enzymes into a stiffer desmoplastic stroma, resulting in higher force distributions that perturb physiological homeostasis for long time scales. Such a persistent increase in tissue rigidity provokes an elevated cytoskeletal tension, driving expression of a malignant phenotype through a force-dependent regulation of integrins [5]. *In vitro* experiments on tumours cells have confirmed that the application of a compressive strain can regulate the expression of genes involved in matrix degradation, cellular adhesion and proliferation [6]. Although the molecular mechanisms of these mechano-transduction pathways have not been fully deciphered, it is known that several signaling cascades (*e.g.* Rho GTPase, FAK, ERK) transmit the altered integrin-mediated tensional signals to pathways controlling cell cycle, thus favouring uncontrolled tumour

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proliferation [7]. Moreover, an autocrine ligand-receptor circuit into the extra-cellular space has been found to sense mechanical stress even without any metabolic alteration in the cell [8]. These research advances in cancer biology have pushed the biomedical community toward the design of micro-engineered experimental techniques for quantifying the mechanical effects on biological systems in pathological contexts [9].

From a clinical viewpoint, the avascular phase of tumour development has been investigated using *in vivo* isolation of spherical tumours, such as the nodal carcinoma [10]. Since the seminal work of Sutherland and coworkers [11], *in vitro* experiments on tumour cells embedded inside an inert matrix of agarose gels, also known as multicellular tumour spheroids, have proved to be effective system models to reproduce the growth characteristics in a tumour-like environment. Classical mathematical approaches have been proposed for modelling the experimental results, mostly focusing on the role played by the nutrient consumption and inhibitor accumulation [12]. When Helmlinger and coworkers reported that a mechanical stress can inhibit spheroid growth regardless of host species, tissue of origin, or differentiation state [13], the role of the tensional state in tumour growth has received a great attention in mathematical modelling. The mechanical feedback acting during growth has been investigated using continuous frameworks, such as multiphase mixtures [14–16], or single-cell models [17, 18]. Although successful in reproducing the biophysical characteristics of tumours at small scales, these approaches fail in modelling a solid-like behaviour of the tumour mass. At scales larger than the cell size, continuum mechanics is more appropriate for modelling the solid stress in tumour cells, whose elastic modulus has been measured at about 6 kPa [19], at the light of thermodynamic restrictions. Even if several continuous models have been proposed [20–22], a major drawback may be nested in the arbitrary definition of phenomenological growth-stress relationships.

In this work, we aim at overcoming existing limitations defining a novel multi-scale treatment of mechano-transduction during avascular tumour growth. In sect. 2 we introduce the balance principles for continuous bodies with varying mass, deriving the thermo-dynamical requirements for coupling growth, solid stress and the diffusion of biomolecules. The theory is employed to model the early avascular development of tumour spheroids. In sect. 3, we extend the theoretical framework for modelling the late development of a heterogeneous tumour, when proliferation is concentrated on an outer surface around an expending core of necrotic cells. Finally, the results are discussed in sect. 4, comparing the theoretical predictions with the stress-dependent growth curves observed *in vitro* for multicellular spheroids.

2 Stress-dependent evolution laws for volumetric growth

In this section we define a continuous theoretical framework to describe the mechano-transduction laws for a soft living material undergoing volume and mass changes. Balance principle for growing materials are introduced, and stress-dependent evolution laws are derived from thermodynamical arguments. The theory is finally employed to model the early growth laws of solid tumour spheroids under elastic constraints.

2.1 Balance principles

Let us consider a mapping $\mathbf{x} = \chi(\mathbf{X}, t)$ describing the deformation of single-phase continuous body from its reference position $\mathbf{X}$ to its actual configuration in $\mathbf{x}$. The volumetric growth inside the material can be modeled using a multiplicative decomposition of the deformation gradient $\mathbf{F}$ [23], so that

$$\mathbf{F} = \partial_{\mathbf{X}}\mathbf{x} = \mathbf{F}_e\mathbf{F}_g, \tag{1}$$

where $\mathbf{F}_g$ represents the local deformation gradient imposed by the volumetric growth of the reference configuration, and $\mathbf{F}_e$ is the elastic counterpart, which ensures compatibility of the overall deformation. Setting $J = \det \mathbf{F}$ as the determinant of the Jacobian of the deformation, the time rate of volumetric changes is described by

$$\dot{J} = J\ \nabla \cdot \mathbf{v}, \tag{2}$$

where $\mathbf{v} = \partial_t \mathbf{x}$ is the physical velocity field.

Let ρ_0, ρ be the density of the body with respect to the reference and actual configurations, respectively, being $\rho_0 = J\rho$. The biological material undergoes a volumetric variation (source or absorption) of mass γ, so that the mass balance reads

$$\partial_t \rho + \nabla \cdot (\rho \mathbf{v}) = \gamma\rho. \tag{3}$$

Using eq. (3) in the absence of volumetric forces acting on the material, the balance of linear momentum takes the following form [24]:

$$\rho\dot{\mathbf{v}} = \nabla \cdot \boldsymbol{\sigma}, \tag{4}$$

where the dot indicates the material time-derivative and $\boldsymbol{\sigma}$ is the Cauchy stress tensor, that must be symmetric for fulfilling the balance of the angular momentum.

Finally, indicating with e the internal energy of the material per unit mass, the balance of the mechanical energy using eqs. (3)-(4) reads

$$\rho\dot{e} = \sigma_{ij}u_{ij} - \nabla \cdot \mathbf{Q} + r, \tag{5}$$

where $u_{ij} = (\partial_j v_i + \partial_i v_j)/2$, $\mathbf{Q}$ is the heat flux, r is the external volumetric heat supply, and Einstein's summation rule on repeated indices applies. The local balances derived in this section will be complemented in the following by thermodynamical arguments that restrict the admissibility of growth evolution laws.

2.2 Entropy inequality and dissipative growth laws

Let η be the entropy per unit mass of the growing material, the Clausius-Duhem form of the second law of the thermodynamics has the following local expression:

$$\rho\dot{\eta} \geq -\nabla \cdot \left(\frac{\mathbf{Q}}{\Theta} + \bar{\mathbf{Q}}\right) + \frac{r}{\Theta}, \tag{6}$$

where Θ is the absolute temperature, and $\bar{\mathbf{Q}}$ represents an extra-entropy flux, which can possibly account for biochemical dissipation inside the body.

The Helmholtz free energy per unit mass of the material is defined as $\Psi = e - \Theta\eta$, so putting together eqs. (5), (6) and substituting eq. (3), the local form of the dissipation inequality reads

$$\rho(\dot{\Psi} + \eta\dot{\theta}) \leq \sigma_{ij}u_{ij} - \Theta\nabla\cdot\bar{\mathbf{Q}} - \frac{\mathbf{Q}}{\Theta}\cdot\nabla\Theta. \tag{7}$$

Recalling the multiplicative decomposition of the deformation gradient in eq. (1), the free energy per mass unit for a growing continuum must depend only on the purely elastic deformation. Therefore, the following material functional dependence for Ψ can be postulated [25]:

$$\Psi = \Psi(\mathbf{F}_e, c_\alpha, \nabla c_\alpha, \Theta), \tag{8}$$

where c_α are scalar internal variables, that might represent the local concentrations of biomolecules or biological signals diffusing inside the material. The dependence of Ψ on ∇c_α is intended to describe the shape regulations in morphogenetic processes based on the ability of cells to measure gradients comparing their own signalling level with those of their neighbours [26].

Substituting eq. (8) in eq. (7), the following constitutive equation for the elastic stress can be derived:

$$\boldsymbol{\sigma} = \rho\mathbf{F}_e\frac{\partial\Psi}{\partial\mathbf{F}_e}\,. \tag{9}$$

The use of a hyperelastic constitutive law for the tumour is known to be a simplification of a more complex rheology, however no viscoplastic contribution is to be expected here because null shear stress follows from the assumption of spherical symmetry [27,28]. Accordingly, the dissipation inequality in isothermal conditions takes the following reduced expression:

$$M_{ij}\left(\dot{F}_g F_g^{-1}\right)_{ji} - \rho\frac{\partial\Psi}{\partial c_\alpha}\dot{c}_\alpha - \rho\frac{\partial\Psi}{\partial(\nabla c_\alpha)}\cdot\nabla\dot{c}_\alpha + \Theta\nabla\cdot\bar{\mathbf{Q}} \geq 0, \tag{10}$$

where $\mathbf{M} = \mathbf{F}_e^{-1}\boldsymbol{\sigma}\mathbf{F}_e = \rho(\partial\Psi/\partial\mathbf{F}_e)\mathbf{F}_e$ is the so-called Mandel stress, which is the stress measure driving the evolution of material growth. The dissipation inequality in eq. (10) gives the thermodynamical direction for the transformation of biochemical energies into the volumetric growth of a living material. Our objective is to propose stress-dependent evolution equations of the growth tensor in the form

$$\dot{\mathbf{F}}_g = f(\mathbf{F}_g, \mathbf{M}, c_\alpha, \nabla c_\alpha, \bar{\mathbf{Q}}, \Theta), \tag{11}$$

where f is a generic function of the given variables such that eq. (10) is automatically satisfied. An application is presented in the following model of the initial phase of avascular tumour growth.

2.3 Application to stress-dependent avascular growth in tumour spheroids

The early stages of tumour development are regulated by the diffusion properties of nutrient factors and waste products through the surrounding host tissue, a process which is called avascular growth. In practice, the solid tumour is made by a small agglomeration of few cells which rapidly proliferate thanks to the large availability of nutrients in the environment while deforming the external healthy stroma. In this paragraph we aim at studying the mechano-transduction characteristics in the avascular growth phase of tumour spheroids applying the proposed theoretical framework. Let us consider the volumetric growth of a tumour spheroid occupying the core nucleus of a healthy surrounding tissue, as depicted in fig. 1. The thermo-mechanical fields related to the tumour mass and the host tissue are indicated in the following by using the superscripts t and h, respectively, using a spherical coordinate system (r, θ, ϕ). We assume that the tumour spheroid undergoes a homogeneous volumetric growth at uniform temperature Θ, such that

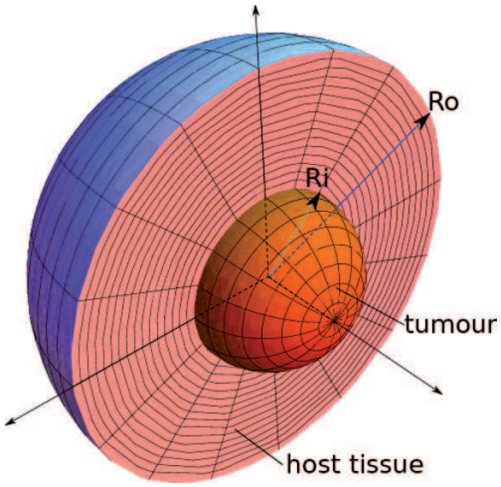

Fig. 1. Scheme of the avascular growth of a tumour spheroid. The tumour mass core is constrained by a surrounding healthy tissue, whose inner and outer radii are indicated as $r_i(t)$ and $r_o(t)$, respectively.

$$(F_g^t)_{ij} = g(M_{ij}^t, t)\delta_{ij}, \tag{12}$$

where δ_{ij} is the Kronecker delta, and $g = g(M_{ij}^t, t)$ represents the time- and stress-dependent avascular growth rate. Recalling that both the tumour cells and the healthy stroma are incompressible media with a density close to that of water, we must impose the incompressibility of the elastic deformations, being $\det\mathbf{F}_e^k = 1$, with $k = (h, t)$. Therefore, additively separating the biochemical and the elastic contribution in eq. (8), a neo-Hookean mechanical behaviour can be postulated so that the free energies of the materials read

$$\Psi^k = \frac{\mu^k}{2}\left[(F_e^k)_{ij}(F_e^k)_{ji} - 3\right] - p^k\left(\det\mathbf{F}_e^k - 1\right) + \Psi_n^k(n, \nabla n), \tag{13}$$

where μ is the shear modulus, p is a Lagrange multiplier ensuring incompressibility, and Ψ_n is the biochemical free energy depending on the nutrient concentration n. Using eqs. (9), (13), the Cauchy stress components inside the

materials read

$$\sigma^k{}_{ij} = \mu^k \rho^k (F_e^k)_{il} (F_e^k)_{jl} - p^k \delta_{ij}, \tag{14}$$

where ρ is the density, that for both materials is very close to water density, and summation rule only applies to repeated subscripts. Considering that the growth process happens at long time scales, eq. (4) in quasi-static conditions reduces to $\nabla \cdot \boldsymbol{\sigma}^k$=0, which in spherical coordinates rewrites

$$(r^2 \sigma^k_{rr})_{,r} - r(\sigma^k_{\theta\theta} + \sigma^k_{\phi\phi}) = 0, \tag{15}$$

where comma denotes partial differentiation. In order to avoid a singularity in $r = 0$, we derive from eq. (15) that the tumour must be subjected to homogeneous deformation and stress states, such that

$$(F_e^t)_{ij} = \delta_{ij}; \qquad \sigma^t{}_{ij} = \rho^t(\mu^t - p^t)\delta_{ij}. \tag{16}$$

Indicating the reference and spatial radii as R and r, respectively, the global incompressibility conditions impose

$$r^t = g \cdot R^t; \qquad r^h = \sqrt[3]{(R^h)^3 + (g^3 - 1)R_i^3}. \tag{17}$$

Substituting eq. (17) in eqs. (14), (15), the radial stress component σ^h_{rr} inside the host tissue reads

$$\sigma^h_{rr} = -2\mu^h \rho^h \int_r^{r_o} \frac{(r^h/R^h)^2 - (R^h/r^h)^4}{r^h} \mathrm{d}r^h, \tag{18}$$

where we used the stress-free boundary condition at the outer radius, $\sigma^h_{rr}(r_o) = 0$. The continuity of the stress at the interface between tumour and host tissue also imposes that $\sigma^t_{rr}(r_i) = \sigma^h_{rr}(r_i)$. Therefore, using eqs. (16), (18), the Mandel stress components inside the tumour reads

$$M^t_{ij} = \frac{\mu^h \rho^h}{2} \left(\frac{4g^3 + 1}{g^4} - \frac{R_o(5R_o^3 + 4(g^3 - 1)R_i^3)}{(R_o^3 + (g^3 - 1)R_i^3)^{4/3}} \right) \delta_{ij}, \tag{19}$$

which represents a spherical homogeneous compression (respectively, tension) for $g > 1$ (respectively, $g < 1$). We now undertake a field-theoretical viewpoint in order to couple the transformation of biochemical energy into avascular tumour growth [29]. Let us assume the following expression for the extra entropy flux $\bar{\mathbf{Q}}^t$ inside the tumour:

$$\bar{\mathbf{Q}}^t = \rho^t \frac{\partial \Psi^t}{\partial(\nabla n)} \frac{\dot{n}}{\Theta}. \tag{20}$$

Substituting eq. (20) in eq. (10), the reduced dissipation inequality within the tumour mass reads

$$3M^t_{rr} \frac{\dot{g}}{g} - \rho^t \frac{\delta \Psi^t}{\delta n} \dot{n} \geq 0, \tag{21}$$

where δ indicates the functional derivative $\delta_n \Psi^t_n = \partial_n \Psi^t_n - (\nabla \cdot \partial_{\nabla n} \Psi^t_n)$. In particular, the biochemical free energy Ψ^t_n of the tumour can be expressed as follows:

$$\Psi^t_n(n, \nabla n, \Theta) = \frac{\tau}{2}(D_n \nabla n \cdot \nabla n + \gamma_n n^2), \tag{22}$$

where τ, D_n, γ_n are positive coefficients which may depend at most on the temperature Θ. The extra-entropy flux in eq. (20) is therefore directed as the nutrient gradient, being positive (respectively, negative) if nutrient concentration locally increases (respectively, decreases) over time. From eq. (21), a dissipative evolution equation for the nutrients reads

$$\dot{n} = -\frac{\delta \Psi^t_n}{\delta n} \Big/ \tau = -\gamma_n n + \nabla \cdot (D_n \nabla n), \tag{23}$$

which is a classical reaction-diffusion equation, where γ_n is the absorption term and D_n the diffusion coefficient inside the tumour. Taking into account very small tumours with characteristic time of nutrient diffusion (few minutes) much smaller than the characteristic time of growth (days), the nutrient density is initially constant everywhere and the early avascular growth is homogeneous. In such conditions, we can make the assumption that the biochemical dissipation rate can be transformed into the creation of tumour mass at a fixed conversion rate ζ, possibly dependent on n, such that

$$\frac{\delta \Psi}{\delta n} \dot{n} = \tau \dot{n}^2 = \zeta \frac{\dot{J}}{J} = 3\zeta \frac{\dot{g}}{g}. \tag{24}$$

Substituting eqs. (19), (24) into the reduced dissipation inequality in eq. (21), we can finally postulate the following stress-dependent evolution law for the avascular tumour growth:

$$\frac{\dot{g}}{g} = K^t \rho^h \left[\zeta + \frac{\mu^h}{2} \left(\frac{4g^3 + 1}{g^4} - \frac{R_o(5R_o^3 + 4(g^3 - 1)R_i^3)}{(R_o^3 + (g^3 - 1)R_i^3)^{4/3}} \right) \right], \tag{25}$$

where K^t is the tumour growth per free energy unit. Equation (25) has been numerically integrated using a fourth-order Runge-Kutta algorithm, and the growth curves are shown in fig. 2. In particular, the numerical results show that the elastic constraint of the healthy tissue imposes a growth rate reduction depending on the aspect ratio R_o/R_i. Using such a mechano-transduction coupling, we find that the elastic compression inhibits the growth of tumour cells even when nutrients are fully available. The predicted evolution laws are in accordance with the early growth kinetics observed experimentally for multicellular spheroids. At later stages of the development, the tumour core becomes necrotic because the nutrient concentration decays under a physiological threshold, and only the outer cells keep proliferating. This phenomenon is out of reach for a single-phase material model, and requires a more sophisticated theoretical framework that will be introduced in the following section.

3 Mechano-transduction laws in hetereogeneous tumours with a growing interface

In this section we will introduce a thermo-mechanical theory for modelling the stress-dependent growth laws in heterogeneous materials. First, we make some preliminary

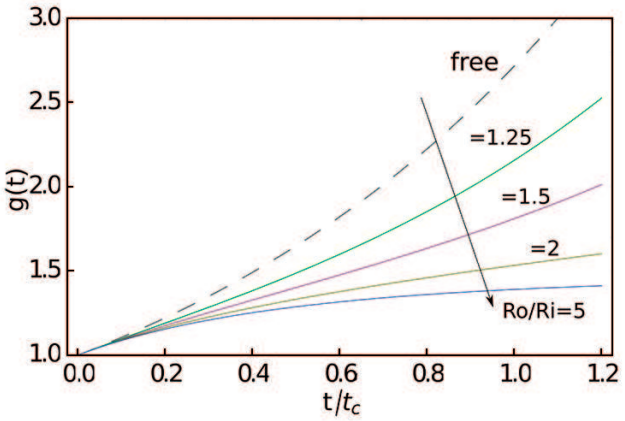

Fig. 2. Evolution of the growth rate g *versus* the dimensionless time t/t_c, where $t_c = 1/(\zeta \rho^t K^t)$ is a characteristic time. The solid lines represent the numerical simulations at the labeled values of aspect ratio R_o/R_i, setting $\mu^h = \zeta K^t$. The dashed curve depicts the exponential free growth ($\mu^h = 0$).

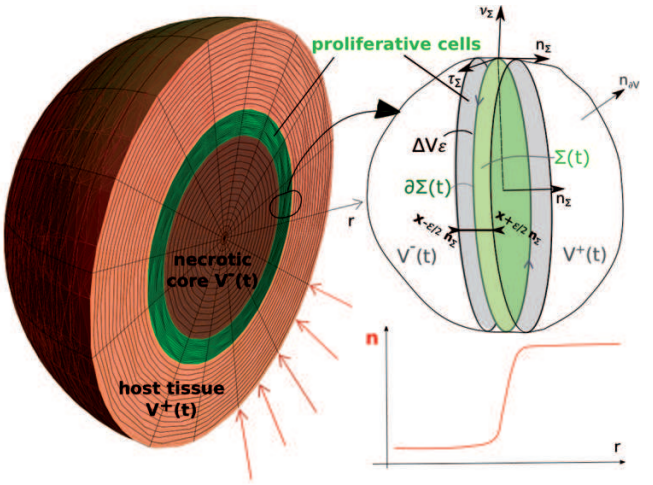

Fig. 3. Section of a heterogeneous multicellular spheroid with a necrotic core and an outer rim of proliferative cells (left). The rim size is fixed by the front width of the diffusive nutrients n, and growth is modeled as a moving non-material interface Σ (right).

assumptions for coupling volumetric growth with mass transport phenomena across boundaries and/or material interfaces. We later define balance laws and thermodynamic principles driving the evolution of homogeneised surface fields carried by a non-material interface. Finally, we apply the proposed theoretical framework to model the avascular growth of a spheroid tumour with a developed necrotic core.

3.1 Definition of the heterogeneous tumour model

In living material growth processes can occur at different length scales, often resulting from a complex local interplay between mass transport phenomena and the diffusion properties of biomolecules. This happens, for example, during embryogenesis: cells duplicate inside narrow regions created by the diffusion fronts of morphogenetic signals, and also rearrange their macroscopic volumes and material positions in order to reach an homeostatic state [30]. In order to describe such a complexity in biological systems, a theoretical framework has been recently proposed for bridging scales in growth modelling [31]. Let us consider the avascular growth of a tumour spheroid which has developed a necrotic core, as depicted in fig. 3. Because of the low concentration of nutrient factors diffusing inside the tumour, cell proliferation is constrained at the tumour border. The size of such a viable cell rim is fixed by the typical diffusion fronts of the nutrients, and it has been measured in multicellular experiments at about 50–250 μm for tumour spheroids of the size of mm and for different concentrations of glucose and oxygen [32]. The proliferative rim occupies a much smaller volume ΔV_ϵ compared to the necrotic core, so that the physical fields inside the tumour mass undergo fast but continuous variation inside ΔV_ϵ. In order to discard such a microscopic variability, we can model the proliferative rim as a moving surface $\Sigma(t)$ with outer normal $\mathbf{n}_\Sigma$. This surface behaves as a non-material interface carrying thermo-mechanical properties, and it is described using a local parametrization expressing the spatial position vector as $\mathbf{x} = \mathbf{x}(u^1, u^2)$, having tangent bases $\mathbf{a}_l = \mathbf{x}_{,u^l}$ with $l = (1, 2)$. The parametric velocity $\bar{\mathbf{v}}_\Sigma$ of the surface can be decomposed as

$$\bar{\mathbf{v}}_\Sigma = \bar{\mathbf{v}}_{\Sigma s} + \bar{v}_{\Sigma n}\ \mathbf{n}_\Sigma, \tag{26}$$

where $\bar{\mathbf{v}}_{\Sigma s}$ is assumed to correspond to the projection of the physical velocity on the surface, whose value depends on the parametrization. In summary, we define an interfacial growth mechanism by defining homogeneised physical fields on the moving surface $\Sigma(t)$, driving the proliferation characteristics of the tumour cells. In order to fulfill the conservation of the thermo-mechanical properties of the biological system, a number of balance principles for the averaged surface fields are introduced in the following.

3.2 Balance principles for the surface fields

Let us consider our biological system as made by two different materials occupying growing adjacent regions $V^-(t)$ (*i.e.* the necrotic core) and $V^+(t)$ (*i.e.* the healthy stroma), separated by the non-material interface $\Sigma(t)$ (*i.e.* the proliferative tumour cells). In the following the superscripts "$-$" and "$+$" will be used for indicating the physical fields inside the volumes having outer normal $\mathbf{n}^- = -\mathbf{n}^+ = \mathbf{n}_\Sigma$, respectively. The volumetric physical fields for the necrotic core and the healthy tissue are subjected to the balance principles discussed in sect. 2.1. The interfacial growth occurs in a very narrow layer of thickness ε, defined as

$$\Delta V_\varepsilon = \bigcup (\mathbf{x} + \nu \mathbf{n}_\Sigma)\,; \quad \forall\, \mathbf{x} \in \Sigma(t), \quad -\varepsilon/2 \le \nu \le \varepsilon/2 \tag{27}$$

so that we can obtain surface fields on $\Sigma(t)$ by homogenization of the volume fields, calculating their finite limit for $\varepsilon \to 0$. Indicating with the subscript Σ such surface physical fields, we can therefore introduce a number of balance principles for the conservation of the thermomechanical properties of the entire system. Dealing with a moving discontinuity, we define the Thomas derivative as $\frac{\delta_t(\cdot)}{\delta_t t} = \frac{\partial(\cdot)}{\partial t} + \bar{v}_{\Sigma n}\mathbf{n}_\Sigma \cdot \nabla(\cdot)$, and we introduce the jump operator $[\![\cdot]\!] = (\cdot)^+ - (\cdot)^-$.

For matters of generality, we assume that generic mass fluxes $\mathbf{m}^-$ and $\mathbf{m}^+$ may exist between the volumes and the moving interface. Therefore, by applying the transport and divergence theorems in a system with a non-material discontinuity, the surface mass balance takes the following form:

$$\frac{\delta_t \rho_\Sigma}{\delta_t t} + \nabla_\Sigma \cdot (\rho_\Sigma \mathbf{v}_{\Sigma s}) - K\rho_\Sigma \bar{v}_{\Sigma n} = \rho_\Sigma \gamma_\Sigma + [\![\rho(\bar{v}_{\Sigma n} - v_n) + \mathbf{n}_\Sigma \cdot \mathbf{m}]\!], \tag{28}$$

where $\nabla_\Sigma \cdot$ is the surface divergence, K is twice the local mean curvature, v_n is the normal component of the physical velocity and γ_Σ is the surface mass source.

Using eq. (28) in the absence of surface external forces, the balance of linear momentum on the surface reads

$$\rho_\Sigma \frac{\delta_t \mathbf{v}_\Sigma}{\delta_t t} + (\rho_\Sigma \mathbf{v}_{\Sigma s} \cdot \nabla_\Sigma)\mathbf{v}_\Sigma = \nabla_\Sigma \cdot \boldsymbol{\sigma}_\Sigma + [\![(\mathbf{v} - \mathbf{v}_\Sigma)(\rho(\bar{v}_{\Sigma n} - v_n) + \mathbf{n}_\Sigma \cdot \mathbf{m}) + \mathbf{n}_\Sigma \cdot \boldsymbol{\sigma}]\!], \tag{29}$$

where $\boldsymbol{\sigma}_\Sigma$ is the Cauchy stress tensor acting on the surface. It is useful to remark that eq. (29) represents the generalization of the Young-Laplace law for a growing non-material interface.

The balance of angular momentum on the surface can be written as

$$\mathbf{a}^l \cdot \boldsymbol{\sigma}_\Sigma \times \mathbf{a}_l = 0, \quad l = (1,2), \tag{30}$$

where $\times$ is the cross-product and $\mathbf{a}^l$ indicate the reciprocal tangent bases. In practice, eq. (30) imposes that $\boldsymbol{\sigma}_\Sigma$ is a tangential field on $\Sigma(t)$ with symmetric surface components.

Defining the internal energy e_Σ for unit mass on the surface, after some manipulations involving eqs. (28), (29) the following conservation law can be derived:

$$\rho_\Sigma \dot{\epsilon}_\Sigma = \mathbf{a}^l \cdot \boldsymbol{\sigma}_\Sigma \cdot \mathbf{v}_{\Sigma,l} - \nabla_\Sigma \cdot \mathbf{Q}_\Sigma + r_\Sigma + \left[\!\!\left[\mathbf{n}_\Sigma \cdot \boldsymbol{\sigma} \cdot (\mathbf{v} - \mathbf{v}_\Sigma) + \left(\frac{(\mathbf{v} - \mathbf{v}_\Sigma)^2}{2} + \epsilon - \epsilon_\Sigma \right) \times (\rho(\bar{v}_{\Sigma n} - v_n) + \mathbf{n}_\Sigma \cdot \mathbf{m}) - \mathbf{n}_\Sigma \cdot \mathbf{Q} \right]\!\!\right]. \tag{31}$$

Using the Gauss-Weingarten formulas with eq. (30), we can prove the useful identity

$$\mathbf{a}^l \cdot \boldsymbol{\sigma}_\Sigma \cdot \mathbf{v}_{\Sigma,l} = \sigma_\Sigma^{lm} \left((\mathbf{v}_{\Sigma s})_{l;m} - \mathbf{v}_{\Sigma n} K_{lm} \right), \tag{32}$$

where ; indicates the covariant derivative on $\Sigma(t)$, and $K_{lm} = -\mathbf{a}_{l,m} \cdot \mathbf{n}_\Sigma$ are the components of the second fundamental form of the surface, with $l, m = (1,2)$.

The Clausius-Duhem form of the second law of thermodynamics for the surface reads

$$\rho_\Sigma \dot{\eta}_\Sigma \geq \frac{r_\Sigma}{\Theta} - \nabla \cdot \left(\frac{\mathbf{Q}_\Sigma}{\Theta} \right) - [\![\mathbf{n}_\Sigma \cdot (\mathbf{Q}/\Theta + \bar{\mathbf{Q}}) - (\eta - \eta_\Sigma)(\rho(\bar{v}_{\Sigma n} - v_n) + \mathbf{n}_\Sigma \cdot \mathbf{m})]\!]. \tag{33}$$

Putting together eqs. (31), (33) and defining a surface free energy Ψ_Σ per unit mass, the dissipation inequality on the surface can be written as

$$\rho_\Sigma \dot{\Psi}_\Sigma \leq \mathbf{a}^l \cdot \boldsymbol{\sigma}_\Sigma \cdot \mathbf{v}_{\Sigma,l} + \left[\!\!\left[\mathbf{n}_\Sigma \cdot \boldsymbol{\sigma} \cdot (\mathbf{v} - \mathbf{v}_\Sigma) - \Theta \mathbf{n}_\Sigma \cdot \bar{\mathbf{Q}} + \left(\frac{(\mathbf{v} - \mathbf{v}_\Sigma)^2}{2} + \Psi - \Psi_\Sigma \right)(\rho(\bar{v}_{\Sigma n} - v_n) + \mathbf{n}_\Sigma \cdot \mathbf{m}) \right]\!\!\right], \tag{34}$$

where we assumed isothermal, uniform conditions for matters of notation compactness. In the following, we use the dissipation inequality in eq. (34) for modelling the mechano-biology of a heterogeneous tumour, defining constitutive equations and growth evolution laws for the proliferative cell rim.

3.3 Application to stress inhibition of a growing tumour rim

Let us first introduce a parametric representation of our proliferative tumour surface $\Sigma(t)$ depicted in fig. 3, using the spherical coordinates (θ, ϕ). The surface position in the reference configuration reads $\mathbf{X}_{\Sigma_0}$, which later moves to the grown spatial position $\mathbf{x}_\Sigma$. According to this parametric representation, the base vectors $\mathbf{a}_l$ ($\mathbf{a}_{l0}$) span the tangent plane of the surface in the actual (reference) configuration, being defined as follows:

$$\mathbf{a}_\theta = r_i \sin\phi \; \mathbf{e}_\theta; \qquad \mathbf{a}_\phi = r_i \; \mathbf{e}_\phi, \tag{35}$$

where $\mathbf{e}_j (j = r, \theta, \phi)$ are the unit vectors of the spherical framework, so that the surface normal reads $\mathbf{n}_\Sigma = \mathbf{e}_r$. Therefore, non-zero metric coefficients in the actual configurations read $g_{\theta\theta} = r_i^2$ and $g_{\phi\phi} = r_i^2 \sin^2\phi$. Considering an axisymmetric deformation, the following useful relations can be derived:

$$g_{\alpha\beta} = (r_i/R_i)^2 \, G_{\alpha\beta}; \qquad K_{\alpha\beta} = r_i^{-1} g_{\alpha\beta}, \tag{36}$$

where $G_{\alpha\beta}(\alpha, \beta = \theta, \phi)$ are the reference metric coefficients. Assuming that the tumour proliferation occurs at a constant reference density $\rho_{\Sigma g} = J_\Sigma \rho_\Sigma$, the free energy per unit mass of the growing rim can be postulated as

$$\Psi_{\Sigma_0} = \varpi \sqrt{(\det g_{\alpha\beta})/(\det G_{\alpha\beta})} = \varpi \; J_\Sigma, \tag{37}$$

J_Σ being the surface Jacobian of the growing surface, so that ϖ acts like a surface tension coefficient. Recalling the following useful relation

$$\dot{g}_{\alpha\beta} = 2\mathbf{a}_\alpha \cdot (\bar{\mathbf{v}}_\Sigma)_{,\beta} = 2((\bar{\mathbf{v}}_{\Sigma s})_{\alpha;\beta} - \bar{\mathbf{v}}_{\Sigma n} K_{\alpha\beta}) \tag{38}$$

and substituting it into eq. (34), the following constitutive equation can be postulated:

$$\sigma_{\Sigma}^{\alpha\beta} = 2\rho_{\Sigma}\frac{\partial\Psi_{\Sigma}}{\partial g_{\alpha\beta}} = \rho_{\Sigma g}\varpi\ g^{\alpha\beta}. \tag{39}$$

From eqs. (32), (39), the reduced dissipation equation for the tumour cell rim rewrites

$$\begin{aligned}&\left[\!\!\left[\left(\frac{(\mathbf{v}-\mathbf{v}_{\Sigma})^2}{2}+\Psi-\Psi_{\Sigma}\right)(\rho(\bar{v}_{\Sigma n}-v_n)+\mathbf{n}_{\Sigma}\cdot\mathbf{m})\right.\right.\\ &\left.\left.+\mathbf{n}_{\Sigma}\cdot[\boldsymbol{\sigma}\cdot(\mathbf{v}-\mathbf{v}_{\Sigma})-\Theta\bar{\mathbf{Q}}]\right]\!\!\right]\\ &+\sigma_{\Sigma}^{\alpha\beta}K_{\alpha\beta}(\bar{v}_{\Sigma n}-v_{\Sigma n})\geq 0.\end{aligned} \tag{40}$$

Let us now assume that the proliferative tumour cells have no mass exchanges with the outer healthy tissue (*i.e.* $\mathbf{m}^{+}=0$), choosing a parametrization such that $v_n^{+}=\bar{v}_{\Sigma n}=\bar{v}_{\Sigma n}$ at any time t. Nevertheless, the rim cells can undergo apoptosis over time so that the necrotic core is constantly growing thanks to a mass flux $\mathbf{m}^{-}$ at the interface $\Sigma(t)$. In particular, we can make the following constitutive assumption:

$$\mathbf{m}^{-}=\rho^{-}(v_r^{-}-\bar{v}_{\Sigma r})\mathbf{e}_r=-\rho^{-}\bar{v}_{\Sigma r}\mathbf{e}_r \tag{41}$$

describing a mass deposition of necrotic cells, having the volumetric density ρ^{-}. From eqs. (28), (41), the mass balance inside the proliferative rim rewrites

$$\frac{\delta_t\rho_{\Sigma}}{\delta_t t}+2\frac{\rho_{\Sigma}\bar{v}_{\Sigma n}}{r_{\Sigma}}=\rho_{\Sigma}\gamma_{\Sigma}, \tag{42}$$

with γ_{Σ} being the tumour mass source on the surface.

Substituting eqs. (39), (41) into eq. (29), the linear momentum balance for the cell rim reads

$$\nabla_{\Sigma}\cdot\boldsymbol{\sigma}_{\Sigma}+[\![\mathbf{n}_{\Sigma}\cdot\boldsymbol{\sigma}]\!]=\left(-2\frac{\rho_{\Sigma g}\varpi}{r_i}+\sigma_{rr}^{+}|_{r_i}-\sigma_{rr}^{-}|_{r_i}\right)\mathbf{e}_r=0, \tag{43}$$

where we assumed that surface growth occurs in quasi-static conditions. Taking into account the constitutive relations in eqs. (14), (17), (18), the stress component $\sigma_{rr}^{+}|_{r_i}$ in the outer tissue at the surface reads

$$\sigma_{rr}^{+}|_{r_i}=\frac{\mu^h\rho^h}{2}\left(\frac{R_i(4r_i^3+R_i^3)}{r_i^4}-\frac{R_o(5R_o^3+4(r_i^3-R_i^3)}{(r_i^3+R_o^3-R_i^3)^{4/3}}\right). \tag{44}$$

Using the same field-theoretical approach as in sect. 2.3, we can assume a positive-definite energy dissipation generated by the extra-entropy flux, defined as

$$\mathbf{e}_r\cdot[\![-\Theta\bar{\mathbf{Q}}]\!]=\kappa_{\Sigma}\frac{\dot{J}_{\Sigma}}{J_{\Sigma}}=2\kappa_{\Sigma}\frac{\bar{v}_{\Sigma n}}{r_i}, \tag{45}$$

so that κ_{Σ} represents the rate of free energy converted into newborn tumour cells. Taking into account eqs. (39), (41), (43), (45) within the reduced dissipation inequality in eq. (40), we can finally define the following stress-dependent evolution law for the growing tumour rim:

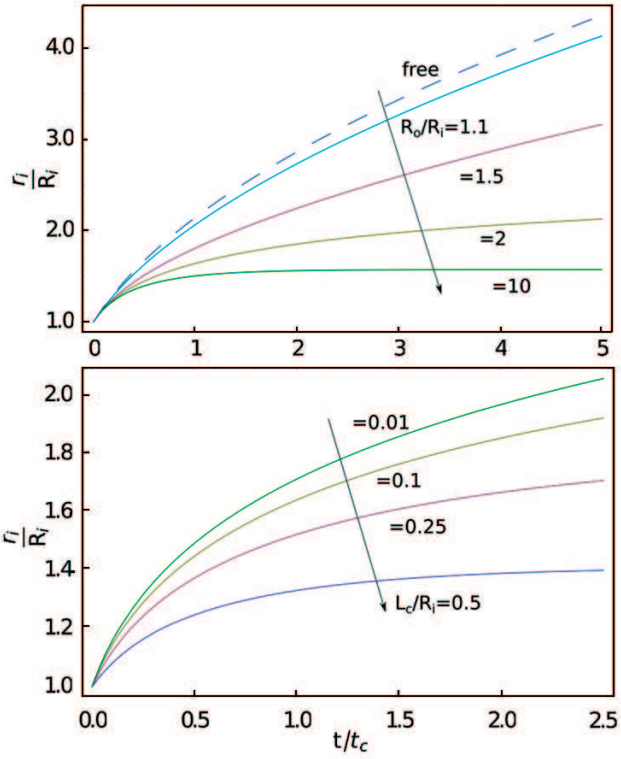

Fig. 4. Evolution of the grown rim radius r_i *versus* the dimensionless time t/t_c. The solid lines represent the numerical simulations at the labeled values of aspect ratio R_o/R_i (top, at $L_c/R_i=0.1$) and of the capillary ratio L_c/R_i (bottom, at $R_o/R_i=2$), setting $\rho^h\mu^h=\kappa_{\Sigma}$. The dashed curve depicts free growth conditions ($\mu^h=0$).

$$\bar{v}_{\Sigma n}=\dot{r}_i=K_{\Sigma}\left(2\frac{\kappa_{\Sigma}-\rho_{\Sigma g}\varpi}{r_i}+\sigma_{rr}^{+}|_{r_i}\right), \tag{46}$$

where K_{Σ} is the rim growth rate per unit free energy. Defining a characteristic time $t_c=R_i/(\kappa_{\Sigma}K_{\Sigma})$, using a tilde upperscore for indicating dimensionless variables eq. (46) rewrites

$$\tilde{\dot{r}}_i=\frac{\dot{r}_i}{R_i}=\frac{1-(L_{\mathrm{cap}}\rho^h\mu^h)/(R_i\kappa_{\Sigma})}{\tilde{r}_i/2}+\tilde{\sigma}_{rr}^{+}, \tag{47}$$

where $L_{\mathrm{cap}}=(\rho_{\Sigma g}\varpi)/(\rho^h\mu^h)$ is a capillary length and $\tilde{\sigma}_{rr}^{+}=\sigma_{rr}^{+}/\kappa_{\Sigma}$. The numerical results of this stress-dependent growth law are depicted in fig. 4 for varying values of the dimensionless parameters L_{cap}/R_i and R_o/R_i. It is useful to remark that the analytical solution of eq. (47) in the case of free boundary (*i.e.* setting $\mu^h=0$) is a capillarity-driven law $\tilde{r}_i\propto\tilde{t}^{1/2}$, representing the diffusive behaviour of freely expanding cell colonies [22]. The stress-dependent growth laws are finally in accordance with experimental results for heterogeneous tumour spheroids. Notably, the numerical curves show that a cross-over effect exists from the diffusive expansion to a stress-saturated growth of the proliferative rim.

4 Discussion and conclusion

In this work, we have investigated the thermo-mechanical bases for coupling volumetric growth and solid stress in tumour growth modelling. Two continuous theoretical approaches have been introduced in order to take into account different mechanisms underlying the avascular growth phase of tumour spheroids. In sect. 2, the tumour mass was modeled as a single-phase continuum whose volumetric growth is regulated by the local concentration of internal variables, representing the diffusive nutrients. After introducing the general balance principles for growing continuous bodies, we have used the Clausius-Duhem form of the entropy inequality to demonstrate in eq. (10) that the Mandel tensor is the stress measure driving the evolution of material growth. Accordingly, we have proposed in eq. (25) a dissipative evolution law for the tumour spheroid, where homogeneous growth is coupled with both the solid stress exerted by the outer healthy stroma and the biochemical energy provided by the nutrient absorbtion. The results depicted in fig. 2 show an exponential expansion in the earliest growth phase, followed by a progressive reduction of the growth rate, which is modulated by the compressive stress inside the tumour mass. Such early growth characteristics are in agreement with classical experimental results on multicellular tumour spheroids. In fact, Freyer and Sutherland have found that tumour spheroids grow exponentially up to a diameter of 150 μm with a doubling time of about 17 h, while further growth rate decreased because of a decreased rate of oxygen and glucose consumption [32, 33]. In practice, the external load drives a progressive accumulation of tumour cells in a quiescent state, which can turn proliferative again if the stress is released, as later observed by Helmlinger *et al.* [13]. Further growth results in the formation of a necrotic core for a spheroid diameter of about 400 μm, due to a reduced penetration length of the nutrients, also caused by a significant increase in the hydrostatic pressure inside the tumour. When the spheroids reach a diameter of about 1000 μm the extent of the necrosis becomes dominant, and proliferation occurs in a narrow outer rim of proliferative cells. Such a heterogeneous tumour is out of reach for a single-phase model, therefore it has been investigated using the multi-scale continuous approach defined in sect. 3. Considering that the tumour growth undergoes fast but continuous variations in a very narrow volume, as shown in fig. 3, we have modelled this surface growth inside the outer rim as a moving non-material interface carrying thermo-mechanical properties. Under this assumption, we have defined a homogeneised surface field on the interface, deriving the required balance principles for the conservation of the thermo-mechanical properties of the entire system. In particular, using the previous thermodynamic approach we have demonstrated in eq. (34) that the surface growth is driven by a jump of the volumetric mechanical energy fluxes across the interface. Assuming that the proliferative cells adhere via a surface tension mechanism in eq. (39), and accounting for a mass flux of dead cells towards the necrotic core in eq. (41), we have derived a stress-dependent surface growth law in eq. (46). The results of this model are shown in fig. 4, for varying geometric and elastic parameters. In particular, we qualitatively recover the typical experimental curves with a cross-over towards a growth saturation regime once reached a limiting solid stress. Such a saturation compressive stress has been measured in multicellular spheroids at about 6–16 kPa in agarose gel experiments [13]. Applying a constant osmotic pressure at around 10 kPa, the duplication rate of bulk cells decreased by a factor 300 [34]. A compressive stress has been also reported to induce tumour cell apoptotis via a mitochondrial pathway [35], therefore playing a key role in determining the homeostatic conditions during avascular tumour invasion.

In conclusion, we have proposed two different theoretical frameworks which successfully modelled the coupled effects of diffusing biomolecules and solid stress inhibition on the avascular growth of tumour spheroid. Using a multi-scale approach and a continuous thermo-dynamical treatment of the growth evolution laws, this work has the potential to provide new insights on the role of microenvironment during tumour invasion. Understanding how the biochemical and the biomechanical forces interact with tumour cells and intervene during intercellular crosstalk is a major challenge for designing patient-specific therapeutic actions for treating cancer.

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