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RESIDUAL STRESS-INDUCED STIFFNESS IN GROWING BIOLOGICAL MEDIA

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Abstract. *The volumetric growth of biological media accounts for the accumulation of residual stresses as the natural consequence of material elastic compatibilization. Stress gradients and internal pressures are known also to directly steer some key biological mechanisms such as nutrient walkways and proliferation progress as well as some physiological processes activated by cells mechanotrasduction. Also, in pathological cases like those of solid tumors, mechanical compression can both induce metastasis and constitute a mechanical hurdle to therapeutic drugs infiltration. As a consequence, evaluation of in situ stresses and the mechanical characterization of biological grown media has become prominent to both furnish important insights in the treatment of some diseases and conceive newly bio-inspired implantable devices mimicking the elastic properties of the host tissue to limit the stress shielding phenomena and drive a favorable process of tissue healing and remodeling. The way in which heterogeneous growth-induced finite stretch and residual stress determine the evolution of tissue properties through the direct modification of material constants is here analyzed by means of a small-on-large approach, in order to capture some relevant mechanical aspects of tissue remodeling, mainly investigating the development of stress-induced anisotropies, growth-driven variations of overall stiffness as well as morphological changes and local instabilities due to growth.*

1 INTRODUCTION

Volumetric growth of biological tissues is generally accompanied by adaptation processes that continually change their structure and affect the evolution of their mechanical properties, also in response to the tissues activity and to the host environment with whom they exchange mechanical as well as physical and chemical stimuli [1]. Mass accretion and structural remodeling, which are treated in biomechanics through finite deformations theory according to the most of the concerning literature [2, 3], can in fact trigger different mechanical and biochemical processes that work in a synergistic manner and allow the biological material to adequately react to mutable environmental conditions. In this sense, soft tissues can be seen as composite materials in which living constituents are responsible for the heterogeneous and dynamical properties, so that the interplay of the growth, remodeling and eventually repair processes with the mechanical stresses becomes critical to discriminate the overall macroscopic behavior and the functionality of the tissue. Among these factors, the accumulation of growth-induced (residual) stress and the evaluation of the grown tissue properties cover a great deal of interest from a biomechanical standpoint in order to characterize material evolution. In fact, residual stress —defined as the resident self-balanced stress field in a free traction body which is typically attributed to the incompatibility of growth deformation [4]— together with the growth-induced compatible deformation promote an alteration of the actual tissue elastic properties. On the other hand, growth-associated stresses can negatively compromise the physiology of the tissue. It is the case, for instance, of solid tumors, within which compression seems to concur in many physiological events associated to their development, such as the formation of hypoxic regions and vascular collapse as well as peripheral migration and lymphangiogenesis [5, 6]. The role of stress gradients in biological media is in fact pivotal in promoting cells reconfiguration and motility and, also steer Fick-type diffusion of chemicals dissolved within the fluid phase as well as macromolecules extravasation throughout the *interstitium*. A wide literature is in fact dedicated to the investigation of tumor mechanical micro-environment in order to establish the nature as well as the causes and the effects of intratumoral residual stresses [4, 7, 8, 5], and are aimed also to propose some mechanically-based hypotheses to prospect intratumoral drug inflow as well as to reduce peritumoral convective flow and thus likely decrease metastasis of cancer cells [9], by also discussing the possibility of mechanically targeting tumor and healthy cells on the basis of their different properties at the single-scale level [10].
Harboring stress in grown tissue can be observed by means of experimental methods [11, 12]. However, experiments to determine the global material response through *ex vivo* mechanical tests on excised tissue samples are not able to reveal in what extent residual stress and adaptive strain affect the intrinsic moduli and the local *in situ* properties. This work therefore focuses on the explicit individuation of the way in which the harboring growth-induced stress and growth deformation actually combine and determine the evolution of the nonlinear behavior of a hyperelastic growing tissue evaluated in terms of its tangent properties, by also including the birth of possible stress-induced heterogeneities and anisotropies. More in detail, the instantaneous elasticity tensor can be evaluated by exploiting a *small-on-large* approach [13] with the aim of mimicking the incremental deformation superimposed on a finitely grown and stressed sample subjected to a mechanical test, and a linear dependence of the tangent moduli upon the Cauchy stress tensor is found, also in accord with some literature formulations [14]. Similar strategies have been adopted, for example, to characterize the kinematic hardening of elasto-plastic materials at finite strains [15] and also in the biomechanical context [16], with reference to the response of hyperelastic vascular structures [17], in which the level of stress can be diriment to

evaluate the structural integrity of the vessel walls to support the *in vivo* pressure regimes [18]. The study of the modification of tangent properties caused by stress also suggests to investigate how the modification of the local properties can affect the overall tissue properties and the mechanical stability of the grown bodies. It is in fact known that growth, and the related stress, can give origin to geometrical instabilities, that could explain malformations during the development of masses in diseases from a purely mechanical standpoint [19]. With reference to the case of tumor spheroids, strategies resorting to infinitesimal perturbation methods or eigenvalue analysis have been applied in the recent literature works [19, 20, 21], to evaluate specific values of homogeneous growth or prestress [21, 19] that can produce overall instability so that a configurational change occurs, leading for example to bi-lobed and multiple-lobed forms. Also, experimental observations ascertain that tumor growth is characterized by macroscopic stiffening [6, 22]. In the present work, the focus is on the determination of the explicit way in which growth affects local tissue properties. In particular, with application to tumor spheroids, we find that growth induces inhomogeneity and anisotropization and, unexpectedly, generates local unstable phases. In this sense, prestressed tumors would represent a biological paradigm of complex elastic composites that can exhibit a negative stiffness phase but overall stability, an unusual behavior of a class of materials that have been argued, at least theoretically, by Drugan and Lakes in some recent works [23, 24, 25]. By then starting from the obtained local behavior, we will give some insights on how these results can influence the tumor overall behavior in terms of global stability and overall stiffening.

2 INCLUDING GROWTH IN FINITE ELASTICITY

The growth of solid bodies can be addressed within the framework of finite elasticity [16, 2, 3]. More specifically, the kinematics of the growing mass is described using the well-established approach of multiple configurations, that provides the multiplicative decomposition of the deformation gradient into the product of distinct tensors, each of them mapping the body material points on a different configuration. To this aim, let $\mathcal{B}_0$ be a body in its reference configuration with volume V_0, as illustrated in Fig 1. The entire deformation of the elastic body is governed by the motion $\mathbf{x} = \mathbf{x}(\mathbf{X}, t)$ that maps the material points $\mathbf{X} \in \mathcal{B}_0$ onto spatial points $\mathbf{x}$ at any time t, so that the overall deformation gradient $\mathbf{F}$ is additionally introduced by accomplishing compatibility with the body particles displacement field $\mathbf{u}(\mathbf{X}, t) \in C^2(\mathcal{B}_0)$, thus giving:

$$\mathbf{F} = \frac{\partial \mathbf{x}(\mathbf{X}, t)}{\partial \mathbf{X}} = \mathbf{I} + \mathbf{u}(\mathbf{X}, t) \otimes \nabla_{\mathbf{X}} \tag{1}$$

where $\otimes$ is the dyadic product and ∇ is the nabla vector, the subscript indicating the coordinates with respect to which the differentiation is performed. The deformation gradient is assumed to be the multiplicative combination of a growth tensor and an elastic deformation due to either adaptation or response to external loads [2, 26]:

$$\mathbf{F} = \mathbf{F}_l \, \mathbf{F}_e \, \mathbf{F}_g \tag{2}$$

In order to analyze the sole effects of finite growth, no external loads are provided, i.e. $\mathbf{F}_l = \mathbf{I}$, so that the classical elastic and growth part of deformation exclusively contribute to mechanical stress. According to this structure, the body first undergoes traction-free growth that maps the reference body material points $\mathbf{X}$ on an intermediate configuration, say $\mathcal{B}_g$ in which they occupy the position $\mathbf{x}_g(\mathbf{X}, t) \in \mathcal{B}_g$. As known, non homogeneous growth can take place in an incompatible manner [4] and elastic deformation is necessary to restore to compatibilize

the independently grown elements by thus exerting suitable self-equilibrated stresses (see Fig 1). Then, the elastic strain $\mathbf{F}_e$ maps the points $\mathbf{x}_g \in \mathcal{B}_g$ onto the actual configuration $\mathbf{x} \in \mathcal{B}_t$ by here accounting the presence of residual stresses. By further assuming isotropic growth one can write $\mathbf{F}_g = \lambda_g \mathbf{I}$ with λ_g representing the growth stretch, also the elastic tensor $\mathbf{F}_e$ results diagonal:

$$\mathbf{F}_e = \mathbf{F}\,\mathbf{F}_g^{-1} \qquad J_e = J J_g^{-1} \tag{3}$$

$J = \det \mathbf{F}$, $J_e = \det \mathbf{F}_e$ and $J_g = \det \mathbf{F}_g$ being the Jacobians of the transformations. In particular, $J_g = dV_g/dV_0 = \lambda_g^3$ represents the volume change due to growth while, following literature suggestions [3], elastic incompressibility –i.e. $J_e = 1$– has been hereinafter assumed. Constitutive assumptions are introduced and, specifically, an incompressible neo-

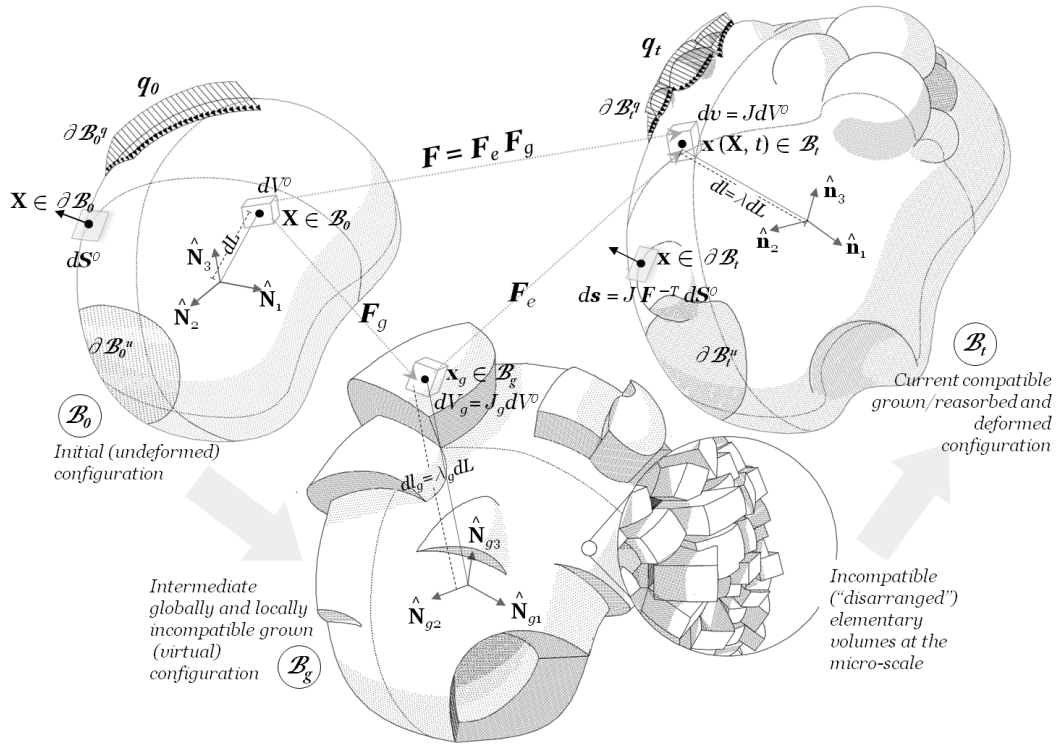

Figure 1: Sketch of the kinematics of growth in finite strain obeying a multiplicative decomposition of the deformation gradient $\mathbf{F}$ into a growth part $\mathbf{F}_g$ mapping material points onto an intermediate and generally incompatible configuration and an elastic part $\mathbf{F}_e$ which drives the body to the current compatible (grown) configuration.

Hookean strain energy density for the tissue has been adopted:

$$\psi_e\left(\mathbf{F}_e\right) = \mu\left(\mathbf{F}_e : \mathbf{F}_e - 3\right) \tag{4}$$

Standard thermodynamic considerations [18] lead to the following expression for the Piola-Kirchhoff (nominal) and the Cauchy (actual) stress in presence of growth:

$$\mathbf{P} = J_g \frac{\partial\,\psi_e}{\partial \mathbf{F}_e}\mathbf{F}_g^{-\mathsf{T}} - J_g\, p\, \mathbf{F}^{-\mathsf{T}} \quad \text{and} \quad \boldsymbol{\sigma} = \frac{\partial\,\psi_e}{\partial \mathbf{F}_e}\mathbf{F}_e^{\mathsf{T}} - p\mathbf{I} \tag{5}$$

with the two stress measure being related through the well-known Nanson's transport formula, i.e. $\mathbf{P} = J\boldsymbol{\sigma}\mathbf{F}^{-\mathsf{T}}$ [27]. Accordingly, the quasi-static balance of linear momentum, by

both supposing vanishing body forces and that the velocity of growth is much slower than the elastic response of the body, is given by [27]:

$$\nabla_{\mathbf{X}} \cdot \mathbf{P} = \mathbf{0}, \quad \forall\, \mathbf{X} \in \mathcal{B}_0 \quad \text{and} \quad \nabla_{\mathbf{x}} \cdot \boldsymbol{\sigma} = \mathbf{0}, \quad \forall\, \mathbf{x} \in \mathcal{B}_t \tag{6}$$

to which opportune traction-type boundary conditions $\boldsymbol{\sigma} \cdot \mathbf{n} = \mathbf{q}_t$ for $\mathbf{x} \in \partial\mathcal{B}_t^q$ or kinematic constraints $\mathbf{u} \cdot \mathbf{n} = \overline{u}$ for $\mathbf{x} \in \partial\mathcal{B}_t^u$ (or, equivalently, their pulled-back versions) are associated.

2.1 Application to a tumor spheroid

The analysis of the finite growth of solid tumor spheroids is a particularly suitable case of interest both from a theoretical and a practical point of view. From a biological standpoint, the growth of solid tumors results from the unpredictable and abnormal proliferation of cells that evaded their natural program. The complex physiology characterizing tumor growth has been far investigated within the field of biomechanics in order to furnish an engineering-based interpretation of the most of biochemical and physical events underlying tumor formation and development. In particular, attention has been focused on the prediction of solid tumor growth and the characterization of growth-induced mechanical stresses, which seem to be actively involved in some adverse mechanisms favoring tumor invasion and opposition to treatment [5]. It is in fact widely accepted that high solid stress and interstitial fluid pressure co-evolve within tumor masses with gradients that cause nutrient outward flow diversion, this orienting tumor peripheral expansion and promoting central necrosis and resistance to drug penetration [9]. With focus on the present application, the relations presented above in general coordinates can be then particularized to study the growth of a tumor spheroid. A spherically symmetric geometry is introduced, so that $\mathbf{X} = \{R, \Theta, \Phi\}$ and the field variables depending exclusively on R. A spherical body is modeled by considering a thick spherical shell with an external radius R_e delimiting the control volume and an inner radius $R_i \to 0$. Furthermore, spherical symmetry ensures that the deformation gradient $\mathbf{F}$ in equation (1) can be conveniently referred to its principal coordinates. By indicating with $\mathbf{x} = \{r, \theta, \phi\}$ the current coordinates, one has:

$$\mathbf{F} = \mathrm{Diag}\{\lambda_r \quad \lambda_\theta \quad \lambda_\phi\} = \mathrm{Diag}\left\{\frac{\partial r}{\partial R} \quad \frac{r}{R} \quad \frac{r}{R}\right\} \tag{7}$$

with $\lambda_\phi = \lambda_\theta$. The elastic deformation, under growth isotropy, hires a diagonal structure and reads:

$$\mathbf{F}_e = \mathrm{Diag}\left\{\frac{\lambda_r}{\lambda_g} \quad \frac{\lambda_\phi}{\lambda_g} \quad \frac{\lambda_\phi}{\lambda_g}\right\} \tag{8}$$

In addition, the linear momentum balance (6) under spherical symmetry returns a sole nontrivial equilibrium equation:

$$\frac{dP_{RR}}{dR} + \frac{2}{R}\left(P_{RR} - P_{\Phi\Phi}\right) = 0, \qquad \frac{d\sigma_{rr}}{dr} + \frac{2}{r}\left(\sigma_{rr} - \sigma_{\phi\phi}\right) = 0 \tag{9}$$

2.2 The effect of heterogeneous growth on *in situ* stresses

To faithfully characterize the stress and stiffness evolution triggered by the growth, the kinematics and the total deformation of a tumor spheroid have been directly related to spherically isotropic growth by exploiting the elastic incompressibility, in a way also to obtain fully analytical results of the nonlinear problem. In fact, by taking into account the gradient form (7), the

constraint equation $\lambda_r\,\lambda_\phi^2 = J_g$ gives the following expression for the deformed radius $r(R)$:

$$r(R) = \left[3\int J_g(\Gamma)\,\Gamma^2\,d\Gamma\right]^{\frac{1}{3}} \tag{10}$$

in which the condition $r(0) = 0$ has been involved. The total stretches have then derived by resorting to relations (7)[1]. Also, for the sake of simplicity, a radially varying growth profile has been assigned. This assumption here neglects the evidences according to which mechanical stress can inhibit in time growth potential [28, 29, 30]. However, here the focus is on the direct effect that growth can have on stress and remodeling of tissue properties, depicted by means of a steady state problem of a freely growing sphere.

2.3 Piecewise constant growth

The simplest case study that allows to analyze the effect of heterogeneous growth within a tissue spheroid can be realized by assigning a piecewise growth profile. To this aim, let R_c be the reference contact radius between an internal spheroid surrounded by an external crown with outer radius R_e. Also, let the volume changes due to isotropic growth be denoted by $J_{g1} = \lambda_{g1}^3$ and $J_{g2} = \lambda_{g2}^3$, respectively. In this situation, equation (10) reduces to $r_1 = \lambda_{g1}\,R$ in the internal sphere, and a hydrostatic stress state $\boldsymbol{\sigma} = \sigma_1\mathbf{I}$ therein occurs, the value σ_1 being given by equation (5):

$$\sigma_1 = \lambda_{g1}^{-2}P_1 = 2\mu_1 - p_1 \tag{11}$$

In the outer shell, by accounting the continuity of displacements at the interface, elastic incompressibility leads to:

$$r_2 = \sqrt[3]{r_c^3 + J_{g2}\,(R^3 - R_c^3)} = \sqrt[3]{J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3} \tag{12}$$

and, consequently, the application of (7) and (8) together with constitutive relations $(5)_1$ let to derive the following expressions for the nominal stress :

$$P_{r2} = 2\mu_2\frac{\lambda_{g2}^4R^2}{(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^{\frac{2}{3}}} - p_2(R)\frac{(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^{\frac{2}{3}}}{R^2} \tag{13}$$

$$P_{\phi2} = 2\mu_2\frac{\lambda_{g2}\,(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^{\frac{1}{3}}}{R} - p_2(R)\frac{\lambda_{g2}^4R}{(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^{\frac{1}{3}}} \tag{14}$$

The linear momentum balance (9) with respect to the reference coordinates in the outer shell, after some passages, leads to the first-order ODE:

$$\frac{dp_2}{dR} = -\frac{4\mu_2\,R_c^6\,\lambda_{g2}\,(J_{g1} - J_{g2})^2}{\sqrt[3]{(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^7}} \tag{15}$$

Direct integration of equation (15) reads:

$$p_2(R) = C_2 - \mu_2\,\lambda_{g2}\frac{R\,(3J_{g2}\,R^3 + 4\,(J_{g1} - J_{g2})\,R_c^3)}{\sqrt[3]{(J_{g2}R^3 + (J_{g1} - J_{g2})\,R_c^3)^4}} \tag{16}$$

[1]It is worth to note that the spherical reference configuration is here assumed residual stretch-free.

The Cauchy stresses of the external phase in accord to relation $(5)_2$ together with the substitution $R = \sqrt[3]{R_c^3(1 - J_{g1}/J_{g2}) + r^3/J_{g2}}$. Then, the integration constant C_2 is obtained by imposing traction-free spheroid growth, i.e. $\sigma_{r2}(r_e) = 0$, where $r_e = r_2(R_e)$ descends from equation (12):

$$C_2 = \frac{\mu_2 \, \lambda_{g2} R_e \left(4R_c^3 \left(J_{g1} - J_{g2}\right) + 5R_e^3 J_{g2}\right)}{\sqrt[3]{\left(R_c^3 \left(J_{g1} - J_{g2}\right) + R_e^3 J_{g2}\right)^4}} \tag{17}$$

the knowledge of the stress in the shell thus allowing to also find the value of the internal pressure p_1.

3 Predicting growth-induced modification of tangent stiffness

The bulk growth of tumor spheroids and the residual stresses associated to the abnormal hyper-proliferation of the mass within a confined region initiate a process of remodeling in which tissue properties dramatically change with respect to the host surroundings. Solid tumors might in fact become very complex materials in a mechanical sense since stress, fluid pressure and mass accretion can combine together in a way to produce tissue heterogeneity and potentially non-standard behavior which, to the best authors' knowledge, has not been discussed. To this purpose, in the light of the very recent biomechanical Literature [19, 21, 20] focusing on growth-induced instabilities, the authors here hypothesize and investigate the possibility for tumor spheroids of exhibiting some unexpected stress-driven characteristics that make them non-ordinary composite materials, showing that residual stress and stretch explicitly affect tissue properties. More specifically, starting from the grown tumor spheroid described in the previous section, the tangent stiffness matrix of the tumor has been evaluated by invoking a *small-on-large* approach. This strategy, whose formulation has been encountered in many classical elasticity works (see for example [14, 13, 31]) and somehow generalizes the well known evaluation of the elastic modulus of a cord under constant tension, provides that a further (small) motion is superimposed to an already finitely deformed body by means of a deformation gradient, say $\mathbf{F}_s = \mathbf{I} + \mathbf{H}_s$, with $\mathbf{H}_s = \mathbf{u}_s \otimes \nabla_{\mathbf{x}}$ being the gradient of the additional displacement $\mathbf{u}_s$ which maps the body points from the position $\mathbf{x}\,(\mathbf{X}, t)$ to the new updated current configuration, as illustrated in Fig 2. In particular, the following expression for the associated tangent stiffness

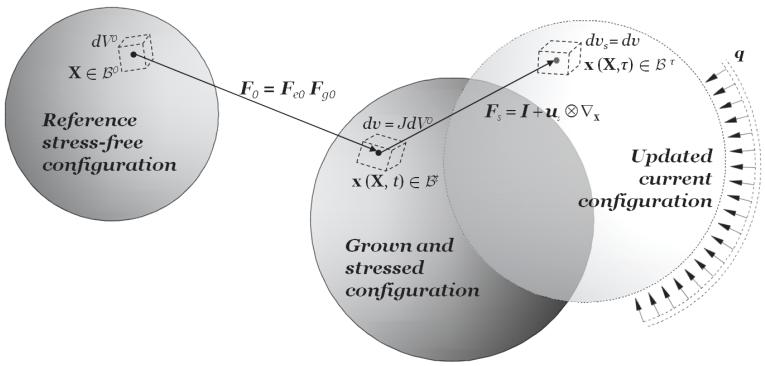

Figure 2: Conceptual scheme of the small-on-large procedure

$\mathbb{C} = [\partial \boldsymbol{\sigma} / \partial \, \mathrm{sym}(\mathbf{H}_s)]_{\mathbf{F}_s \to \mathbf{I}}$ has been derived:

$$C_{ijkl} = \frac{1}{2}\left(\delta_{ik}\sigma_{jl} + \delta_{jl}\sigma_{ik} + \delta_{il}\sigma_{jk} + \delta_{jk}\sigma_{il}\right) + J_e^{-1} \sum_{m,n,p,q} \left(F^e_{im}F^e_{jn} + F^e_{in}F^e_{jm}\right) \frac{\partial^2 \psi}{\partial C^e_{mn} \partial C^e_{pq}} \left(F^e_{pk}F^e_{ql} + F^e_{pl}F^e_{qk}\right) \tag{18}$$

which provides the elastic moduli to linearly depend on the resident stress, while elastic deformation affect the initial moduli in a nonlinear manner.

By accounting the neo-hookean constitutive law (4), the remodeled stiffness tensor of the tumor spheroid exhibits a growth-induced transverse isotropy, the independent nonzero components in this case reading:

$$\begin{aligned} C_{rrrr} &= 2\mu\, \lambda_{er}^2 + 2\sigma_r, \\ C_{\phi\phi\phi\phi} &= 2\mu\, \lambda_{e\phi}^2 + 2\sigma_\phi, \\ C_{r\phi\, r\phi} &= 8\mu \frac{\lambda_{er}^2\, \lambda_{e\phi}^2}{\left(\lambda_{er} + \lambda_{e\phi}\right)^2} + \sigma_r + \sigma_\phi \end{aligned} \tag{19}$$

4 GROWTH-INDUCED STRESS AND MODULI REMODELING

With reference to the problem described in section 2.3, we focus on the modeling of an internal tumor inclusion undergoing homogeneous bulk growth $J_{g1} > 1$, surrounded by a host crown in a homeostatic state, therefore we conveniently set $J_{g2} = 1$ in a way to analyze the effects of mass growth both in terms of internal remodeling and in terms of the environmental response. Furthermore, the two regions are assumed to share the same initial modulus μ. Under these assumptions, and also accounting kinematic equation (12), tumor volume fraction results a sigmoidal function of the growth stretch:

$$\varphi = \varphi_0 \, \frac{J_{g1}}{1 + \varphi_0 \left(J_{g1} - 1\right)} \tag{20}$$

where φ_0 is the initial tumor fraction, see Fig 3. Tumor invasion is accompanied by the accumulation of intratumoral pressure. In particular, as shown in Fig 3, tumor compression increases for lower fractions and growth rates due to the exchange of mechanical forces with a wider environment. Also, the progression of tumor radius allows the mass to win the reaction of the external environment and minimize the built-in stress, the growth *de facto* inducing a pseudo-relaxation of the internal spheroid towards a homogeneous unstressed state as $\varphi \to 1$ within the control volume. The stress state in the overall spheroid, reported in Fig 4A, develops in a way that intratumoral compression is accompanied by the push of the environment in radial direction with circumferential peritumoral tensions that let the host crown accommodate tumor expansion, this trends qualitatively meeting the most of recent literature works [8, 32].

Growth-induced stretch, residual stress actually combine in order to determine the tangent updated properties of the tumor-host spheroid according to expressions (19), whose behavior is illustrated in 4B for fixed growth and starting fraction. Residual stress clearly drives growth-induced transverse isotropy and material inhomogeneity within the host shell, while intratumoral hydrostatic state and homogeneous deformation actually preserve an isotropic remodeling. However, it clearly emerges that harboring stress can induce negative stiffness phases. This feature can occur, for example, when a body that stored (or received) energy is deformed [33], and, consequently, also in presence of stress generated by growth. As a result, residually stressed tumor-host systems can be seen as a complex composite material that exhibits local

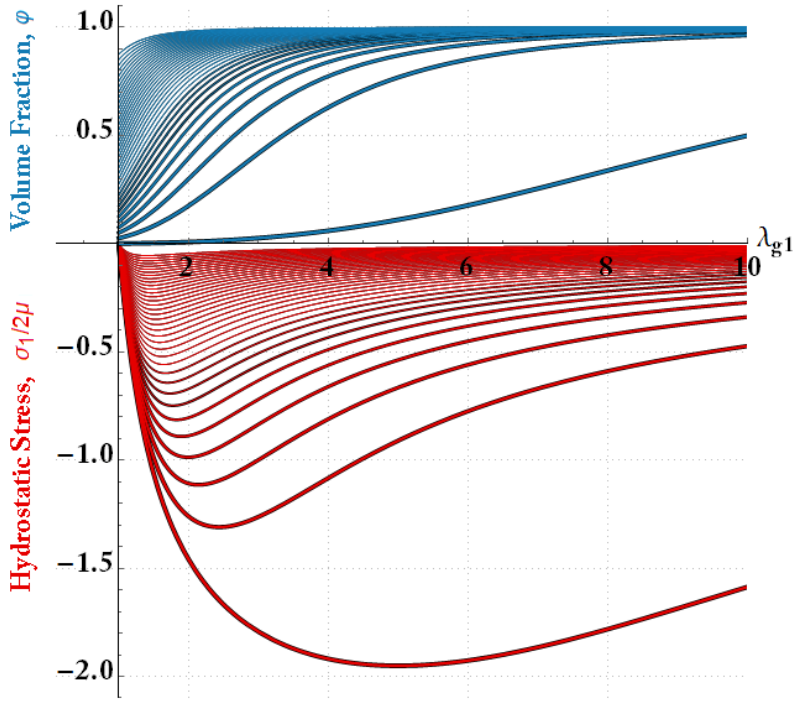

Figure 3: Tumor volume fraction (*top*) and hydrostatic stress (*bottom*) as a function of the growth.

instability due to negative-definite tangent matrix of tangent moduli. The study of the behavior of biphasic composites with a negative stiffness inclusion has been recently carried out by the Drugan and Lakes [24, 33]. In their works, they theoretically postulated the presence of an isotropic and homogeneous internal sphere with negative Young modulus and investigated the conditions ensuring global mechanical stability in terms of homogenized bulk modulus.

Differently, for the case under exam, the alone-unstable phase has been obtained *a posteriori* as the result of the tissue growth and material remodeling, and the inhomogeneous moduli exhibit a transversely isotropic structure. The negative-bulk modulus tumor region is strictly governed by the behavior of the hydrostatic stress due to growth and also depends on the initial tumor volume fraction, in the sense that a strongly confined tumor (in terms of occupied domain) experiences higher pressures (Fig 3) and therefore negative stiffness cores are therein more likely to occur, as also shown in Fig 5A. On the other hand, the tangent matrix of the environmental shell is characterized by a positive circumferential modulus, that can exhibit strong gradients dominated by the behavior of interface hoop stress (see e.g. Fig 4B), while the longitudinal modulus presents a point of zero crossing, say r_0, which denotes a transition between a locally unstable and a stable region and migrates as a function of growth with an approximatevily linear profile reported in Fig 5B.

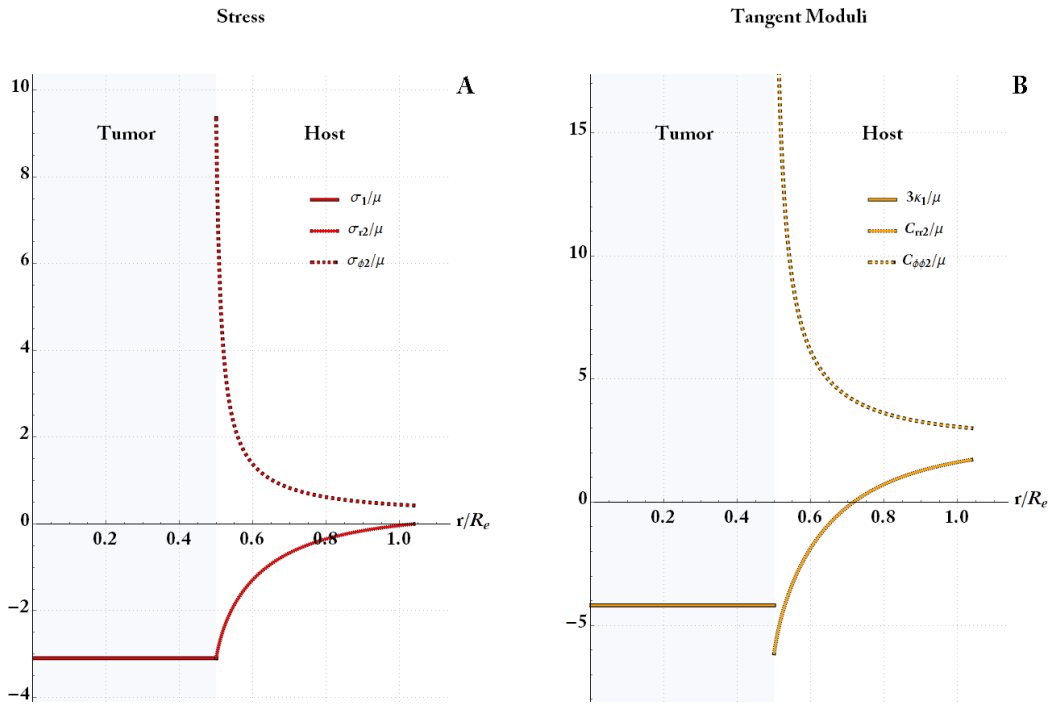

Figure 4: A. Radial and circumferential stress in the tumor spheroid model. B. Development of the tangent moduli, for $\lambda_{g1} = 2.5$ and $R_c = R_e/5$.

5 CONCLUSIONS AND PERSPECTIVES

Solid stress and tissue stiffness affect tumor growth and invasiveness and can have important implications in treatment [6], especially in the study of a mechanical strategy of tumor targeting that exploit the different properties exhibited by cancer and healthy cells [10]. Although *ex vivo* overall properties of biological samples can be measured using a wide range of techniques and, in particular, increased tumor stiffness is a widely accepted biomechanical property [6], the understanding of the way in which *in vivo* local properties associated with growth evolve and the study of the mechanical causes that concur to produce macroscopic tissue stiffening is still partially unclear. The present work presents a mechanical based hypothesis ascertaining that growth and related stress can explicitly determine unusual material inhomogeneities and anisotropization within the tumor tissue, in addition providing the formation of internal unstable phases with negative moduli. This phenomenon connotes residually stressed tumors as a biological paradigm of a special class of (artificial) composites exhibiting such uncommon properties [33]. The presence of an unstable core can be prodromal to global instability. However, the works by Lakes demonstrate that these types of material can be not only overall stable but can also manifest a considerable enhancement of homogenized bulk modulus [33, 24]. In line with these literature evidences and with the above discussed results, we hypothesize that tumors, and in general tissues undergoing growth, can exhibit an analogous behavior and can represent a particular class of biological materials with peculiar local properties that can explain some macroscopic observations still representing an open issue both from a theoretical and an experimental standpoint. In this sense, we aim to orient our future analyses to evaluate the homogenized response of tumor masses in order to: 1) establish an analytical strategy to evaluate the global stability region of tumor growth, 2) investigate the possibility of obtaining

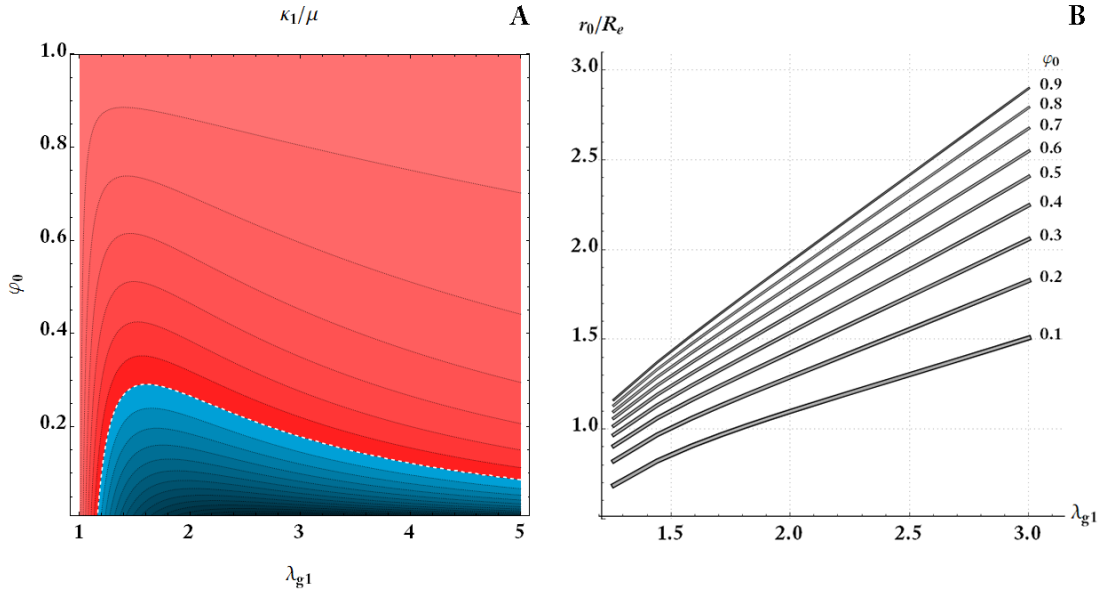

Figure 5: A. Variation of the sign of the tumor bulk modulus and B. Progression of the unstable host region as a function of the growth stretch and the initial volume fraction.

tumor overall stiffening as the effect of the homogenized response of globally stable spheroids at different growth stages and 3) exploit the knowledge of tumor local properties to support the development of mechanically-based strategies for attacking tumor masses.

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