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A PARAMETRIC STUDY OF A MULTIPHASE POROUS MEDIA MODEL FOR TUMOR SPHEROIDS AND ENVIRONMENT INTERACTIONS

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Abstract. *Computational models for tumor growth provide an effective in silico tool to investigate the different stages of cancer growth. Recently, a series of computational models based on porous media theory has been proposed to predict tumor evolution and its interactions with the host tissue. In addition, a specialization of the original models, adapted for tumor spheroids, has been proposed and validated experimentally. However, due to the complexity of the modeling framework, a systematic understanding of the role of the parameters governing the equations is still lacking. In this work, we perform a parametric analysis on a set of fundamental parameters appearing in the model equations. We investigate the effects of a variation of these coefficients on the spheroid growth curves and, in particular, on the final radii reached by the cell aggregates in the growth saturation stage. Finally, we provide a discussion of the results and give a brief summary of our findings.*

1 INTRODUCTION

Cancer is one of the leading causes of the death in the world [1], involving primarily altered cell proliferation and migration of cancer cells to form metastasis in different regions of the body [2]. Recently, it has become clear that a collective effort from all the physical sciences is required to improve our understanding of this illness and design new strategies for therapeutical treatments [3–6]. The development of effective computational models to investigate the growth of a tumor mass is certainly a valid contribution to this field. Mathematical models can analyze the tumor evolution in detail, with a strict control of the parameters governing the equations. Moreover, they can help the experimental investigators in dissecting the dynamics of the systems under study and guide the design of new experiments [7].

In [8], the authors present a computational model for avascular tumor growth based on porous media theory. They apply their modeling framework to the growth of a tumor spheroid *in vitro* and confined in a healthy tissue, where the relative adhesion of tumor cells and host cells to the extracellular matrix is analyzed. Moreover, they study a tumor cord in a three dimensional geometry, where tumor cells grow around microvessels carrying nutrients. They further develop the existing model in [9], where different adhesion interactions between host cells, tumor cells and interstitial fluid are taken into account. Five different cases are dealt with, where interfacial tensions between the cellular constituents and the interstitial fluid are varied, together with their dynamic viscosities. In [10], the authors relax the hypothesis of a rigid extracellular matrix in the tumor tissue, and investigate its deformability making use of rate-dependent plasticity. They apply this framework to analyze the growth of a tumor spheroid in a decellularized extracellular matrix and then in the presence of host cells. Further, they apply the model to study the growth of a melanoma and investigate its temporal and spatial evolution. Another development of the model is given in [11], where the authors introduce remodeling of the extracellular matrix during tumor growth and cell lysis. They study the effect of matrix remodeling on spheroid growth inside a decellularized matrix by comparing their results to the previous implementation. They investigate also the impact of lysis in the dynamics of the system. In [12], the authors specialize the previous modeling framework for tumor spheroids. They carry out a set of experiments on U-87 spheroids to validate the equations and test new constitutive relations. Comparison with experiments is performed both with spheroids freely suspended in a culture medium and subjected to different mechanical loads.

Due to the complexity of these models, an understanding of the role of the different parameters is difficult. For example, in [12] it is not clear which model parameters have a significant effect on the growth of the spheroids. In this paper, we perform a parametric analysis on a set of governing coefficients appearing in the model equations. We test the effect of parameter variation on the spheroid growth curve and in particular on the final radius reached by the cell aggregate. Finally, we provide a discussion of the results and summarize our findings in the conclusions.

2 THE MATHEMATICAL MODEL

The governing equations of the model are derived in the context of porous media theory. Starting from microscopic relations between the constituents, the theory makes use of suitable spatial and temporal averaging theorems to provide balance laws at the scale of the tissue (also termed, “the macroscale”). In this way, the complexity arising from the high spatial variability and several interactions characterizing the microscale is overcome. Then, the introduction of a suitable set of constitutive relations into the macroscale equations provides

the closed form of the problem. Detailed information about the model derivation can be found in previous works of the authors [8–12].

We model the tumor tissue as a biphasic porous medium, composed of the following constituents, or phases: (i) the tumor cells (TCs), which are divided into living (LTCs) and necrotic (NTCs) cells, and (ii) the interstitial fluid (IF), represented in Figure 1. In the language of porous media theory, the union of TCs and extracellular matrix (ECM) constitutes the solid skeleton of the system, whereas the IF represents the fluid phase permeating the pores. The IF carries nutrients, growth factors and waste products; for the sake of simplicity, we consider only one nutrient in our model, namely oxygen (ox), which can diffuse and be consumed by LTCs. Adequate levels of nutrient are necessary for cell proliferation, otherwise they start necrosis and lysis. Finally, we assume cell duplication to be influenced by the local level of mechanical stress, with cells proliferating poorly when subjected to compression. In the following, *t* (tumor) and *l* (liquid) will denote quantities related to the solid and fluid part of the biphasic system, respectively.

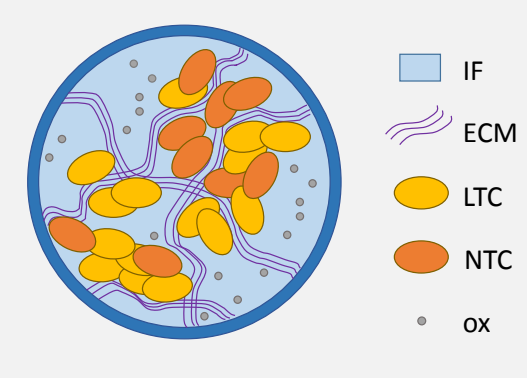

Figure 1: The constituents of the biphasic system.

2.1 The governing equations for tumor spheroids

We denote the volume fractions of the solid and the fluid by ε^t and ε^l, respectively. We assume that the fluid permeates completely the voids left by the solid skeleton, and apply the saturation constraint:

$$\varepsilon^t + \varepsilon^l = 1 \tag{1}$$

Then we write the governing equations for the tumor volume fraction, the interstitial fluid pressure (p^l), the oxygen mass fraction (ω^{ox}) and the necrotic cell mass fraction (ω^{Nt}). In the following, we report the balance laws as appear for the tumor spheroid growth case. We refer the interested reader to [12] for a full derivation. We solve the system of equations given by:

$$\frac{\partial \varepsilon^t}{\partial t} - \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \varepsilon^t \frac{k}{\mu^l} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{\rho}\left(\underset{growth}{\overset{l\to t}{M}} - \underset{lysis}{\overset{t\to l}{M}} \right) = 0 \tag{2}$$

$$\frac{\partial \left(\omega^{Nt} \varepsilon^t \right)}{\partial t} - \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \varepsilon^t \omega^{Nt} \frac{k}{\mu^l} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{\rho}\left(\varepsilon^t r^{Nt} - \underset{lysis}{\overset{t\to l}{M}} \right) = 0 \tag{3}$$

$$\frac{\partial \left[\left(1-\varepsilon^t \right) \omega^{ox} \right]}{\partial t} + \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 \varepsilon^t \omega^{ox} \frac{k}{\mu^l} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{r^2}\frac{\partial}{\partial r}\left[r^2 \left(1-\varepsilon^t \right) D^{ox} \frac{\partial \omega^{ox}}{\partial r} \right] + \frac{1}{\rho} \underset{oxygen}{\overset{ox\to t}{M}} = 0 \tag{4}$$

where r is the radial coordinate over the spheroid radius; k is the intrinsic permeability of the solid matrix; μ^l is the dynamic viscosity of the IF; D^{ox} is the diffusion coefficient of oxygen; and ρ is the density of the phases. As described in detail in [12], Σ is a quantity relating the mechanical stress in the tumor to the solid volume fraction. In the equations above we make use of Σ', the derivative of Σ respect to ε^t, which can be computed analytically from the expression reported in [12].

The mass exchange terms appearing in equations (2)-(4) have the form:

$$\underset{growth}{\overset{l\to t}{M}} = \gamma_g^t \left\langle \frac{\omega^{ox} - \omega_{crit}^{ox}}{\omega_{env}^{ox} - \omega_{crit}^{ox}} \right\rangle_+ H\left(\mathrm{t}_{\mathrm{eff}}^t\right) \omega^{Lt} \varepsilon^t \tag{5}$$

$$\underset{lysis}{\overset{t\to l}{M}} = \lambda_l^t \omega^{Nt} \varepsilon^t \tag{6}$$

$$\varepsilon^t r^{Nt} = \gamma_n^t \left\langle \frac{\omega_{crit}^{ox} - \omega^{ox}}{\omega_{env}^{ox} - \omega_{crit}^{ox}} \right\rangle_+ \omega^{Lt} \varepsilon^t \tag{7}$$

$$\underset{oxygen}{\overset{ox\to t}{M}} = \gamma_0^t \frac{\omega^{ox}}{\omega^{ox} + c^{ox}} \omega^{Lt} \varepsilon^t \tag{8}$$

Here γ_g^t, λ_l^t, γ_n^t and γ_0^t are coefficients that account for the nutrient and IF mass that becomes tumor due to cell growth; the degradation of cellular membranes and the following mass conversion into IF; the rate of cell death; and the oxygen uptake rate in the tumor, respectively. The quantity $\omega^{Lt} = 1 - \omega^{Nt}$ represents the mass fraction of LTCs, and guarantees that growth, death and oxygen uptake are active only on the living portion of the spheroid. The Macaulay brackets $\langle \cdot \rangle_+$ appearing in equations (5) and (7) return the positive value of their argument. Since the oxygen mass fraction inside the spheroid is equal or smaller than its environmental level in the culture medium ω_{env}^{ox}, the brackets in equation (5) will return a value between unity (for $\omega^{ox} = \omega_{env}^{ox}$) and zero (for $\omega^{ox} \le \omega_{crit}^{ox}$). Note that here ω_{crit}^{ox} is the oxygen threshold level below which cell proliferation is inhibited. Equation (6) describes cell lysis occurring in the NTCs, whereas a consideration similar to the one for equation (5) holds true for equation (7), which describes TC death due to the lack of nutrient. Finally, equation (8) describes the uptake of oxygen by LTCs and accounts for the dependence of nutrient consumption on its local level. Here c^{ox} is the oxygen mass fraction at which oxygen consumption is reduced by half. Note that the function H in equation (5) describes the inhibition of cell proliferation due to the mechanical stress exerted on the TCs. Even though several alternatives are given in the literature, in [12] we provide a mathematical expression for this quantity that is able to describe accurately our experimental observations on spheroid growth under a controlled external compression.

We model the growth of the spheroid as a free-boundary problem, where the interface constituted by the TCs moves with velocity v^t, given by:

$$\frac{dR}{dt} = v^t = -\frac{k}{\mu^l} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \tag{9}$$

with R the external radius of the spheroid. The closed form of the differential problem is obtained by defining a set of boundary and initial conditions. In particular, symmetry requires no-flow boundary conditions at the spheroid center, while we assume Dirichlet boundary conditions on the tumor external surface:

$$\frac{\partial \varepsilon^t}{\partial r} = \frac{\partial \omega^{Nt}}{\partial r} = \frac{\partial \omega^{ox}}{\partial r} = 0, \quad \text{in } r = 0, \tag{10}$$

$$\varepsilon^t = \varepsilon^t_{ext}, \quad \omega^{Nt} = 0, \quad \omega^{ox} = \omega^{ox}_{env}, \quad \text{in } r = R \tag{11}$$

Finally, we assume the following initial conditions over the spheroid radius:

$$\varepsilon^t = \varepsilon^t_{ext}, \quad \omega^{Nt} = 0, \quad \omega^{ox} = \omega^{ox}_{env}, \quad \text{on } 0 < r < R \text{ at } t = 0 \tag{12}$$

3 RESULTS AND DISCUSSION

In [12], we have performed numerical simulations of the equations in (2)-(4) and we have recorded the resulting growth curves, namely the evolution of the spheroid radius over time. We have analyzed both the case of spheroids freely growing in their culture medium, and the case where an external compression is applied. The values of the governing parameters are obtained from the literature, when they are available, and from the fit of the experimental curves. In this work, we investigate the dependence of the growth curves on a set of these parameters, summarized in Table 1.

Parameter	Description	Reference value	Unit
R_0	Initial radius of the spheroid	145	μm
ω^{ox}_{crit}	Critical mass fraction of oxygen	2.0×10^{-6}	(−)
γ^t_g	Coefficient related to growth	5.4×10^{-3}	$\mathrm{kg}/(\mathrm{m}^3\cdot\mathrm{s})$
γ^t_n	Coefficient related to necrosis	1.5×10^{-1}	$\mathrm{kg}/(\mathrm{m}^3\cdot\mathrm{s})$
λ^t_l	Coefficient related to lysis	1.15×10^{-2}	$\mathrm{kg}/(\mathrm{m}^3\cdot\mathrm{s})$
α	Coefficient in the definition of Σ	10^5	Pa

Table 1: Parameters considered in this work. The reference value is the one used in [12].

We start our analysis from R_0, the initial radius of the spheroid. In Figure 2 we report the behavior of the growth curve for different initial spheroid radii. Note that in all the following figures the curve in red is the one corresponding to the reference value in Table 1. We consider spheroids with initial radii of 90, 117.5, 145, 172.5 and 200 μm. For all the different conditions, in particular for the small initial radii, it is possible to visualize the three stages characterizing the growth of the spheroids [13]: the exponential phase in the first days of growth, where the cells proliferate in a nutrient-rich environment; the linear phase, where the the tumor mass becomes larger and the nutrient starts to run low; and the growth saturation phase, where a significant portion of the spheroid is necrotic and only a small rim of cell proliferates at the tumor border. Notably, although the spheroids with the larger initial radii are constitued by more tumor cells than the others, they reach a similar final radius, of about 475 μm. This behavior is consistent with our assumption of growth as limited by nutrient deprivation. For a fixed level of external oxygen (ω^{ox}_{env}), only a fixed number of TCs is allowed to coexist in the spheroid mass. This condition is met sooner for the spheroids with larger initial radii and later for the others, as shown in the curves and observed experimentally in [12].

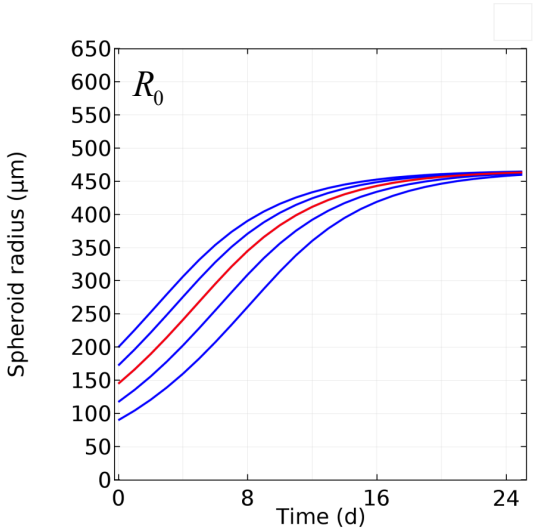

Figure 2: Spheroid growth curves for different initial radii.

Next, we study the effect of a variation in the critical level of oxygen ω_{crit}^{ox}. The resulting growth curves are shown in Figure 3. We consider values for ω_{crit}^{ox} of 1.0×10^{-6}, 2.0×10^{-6}, 3.0×10^{-6}, 4.0×10^{-6}, 5.0×10^{-6} and 6.0×10^{-6} (the black arrow points in the direction of increasing values of ω_{crit}^{ox}; this holds true also for the following figures). The choice of this parameter affects significantly the final radius reached by the spheroids. In particular, higher values of the critical level of oxygen provide smaller final radii. This follows from the modeling choice in equation (5), where cell proliferation is a linear function of the oxygen critical level. If this threshold value is high when compared to the external mass fraction of oxygen, only a small fraction of the spheroid is able to proliferate and the final radius is reduced.

The next parameter that we consider is the growth coefficient γ_g^t. In Figure 4, we consider values of this coefficient that are ±25, ±50 and ±75% of the reference value. Also for this parameter, the final radius reached by the spheroid strongly depends on its value. Interestingly, γ_g^t seems to regulate the time scale of the phenomenon. Actually, for small values of the growth coefficient, at the end of the simulations the spheroid is still in the first stages of growth. On the contrary, for higher values of γ_g^t the spheroid reaches faster its final radius. We also observe that the steady radius increases for increasing values of the growth coefficient. In fact, growth saturation is established when the net production of new tumor mass is zero. This means that, in a given time interval, the number of new cells produced by growth has to be equivalent to the number of cells undergoing lysis. This number is approximately given by the number of necrotic cells times the lysis rate. If the lysis rate is fixed, as in the present case, and the growth coefficient is increased, there is a net production of TCs and the radius grows further. To reach a new steady condition, the necrotic core of the spheroid has to increase in size, so that the number of cells undergoing lysis is again equal to the number of generated TCs. This idea can be tested through a variation of the parameters that regulate cell death, namely γ_n^t and λ_l^t, together with a consistent variation of the growth coefficient γ_g^t. If we double each of these constants at the same time, we expect the steady radius of the spheroid to be unaltered, since we have maintained the original ratio between cell production and removal. This condition is shown in Figure 4, by the gray dashed line. Note that the final radius is the same of the reference value, but the time needed to reach the steady state is significantly reduced.

Then, we consider the case of a variation in the coefficient γ_n^t, regulating the necrosis of the LTCs. The influence of this parameter is shown in Figure 5. For this coefficient, we ana-

lyze values that are ±25, ±50 and +75% of the reference value. As shown in the figure, the variation of γ_n^t has a very little effect on the resulting growth curves. The shape of the curve is not significantly altered and the final radius has a variation of less than 5%. This is probably due to the fact that this term is active on a population of TCs that is still alive, but it is located in a region of the spheroid where the nutrient level is below the critical threshold.

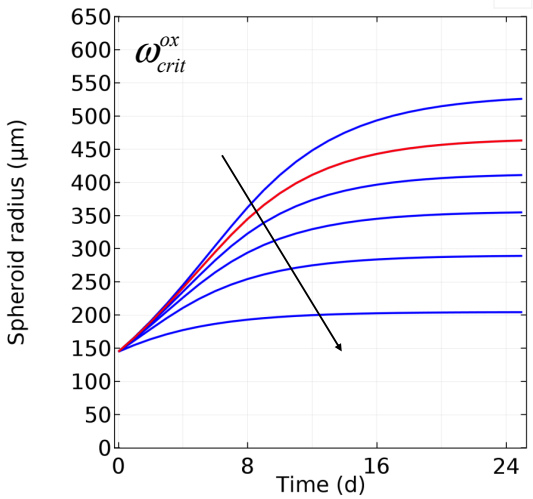

Figure 3: Spheroid growth curves for different critical levels of oxygen.

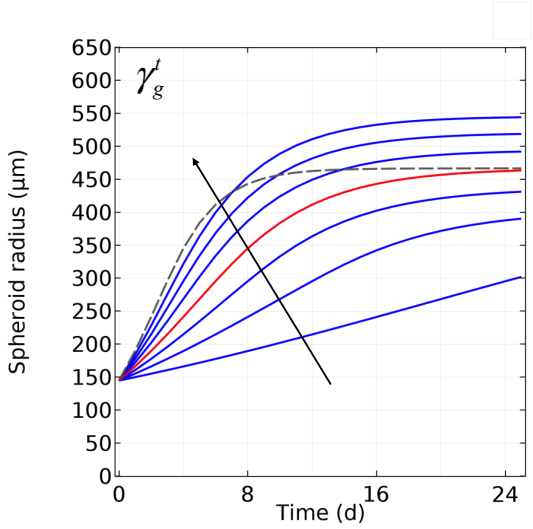

Figure 4: Spheroid growth curves for different values of the growth coefficient.

This condition is poorly encountered in the spheroids at this stage of their growth [14], resulting in the small influence of this parameter.

The fifth parameter that we study is the lysis coefficient λ_l^t. Figure 6 shows the results of considering values for this parameter that are ±25, ±50 and ±75% of its reference value. We first observe that the effects of varying the parameter only appear after day 8 in the simulation. This is consistent with the onset of a necrotic population inside the spheroid, which occurs after the first days of the simulation. These results show that the value of the lysis coefficient has a significant impact on the spheroid final radius. Notably, there is a saturation effect for high values of λ_l^t. This may be due to the limited amount of NTCs that exists in the necrotic core at fixed γ_n^t, and that can therefore undergo lysis.

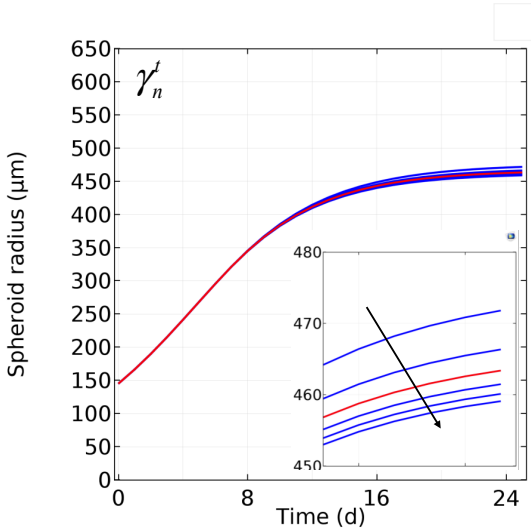

Figure 5: Spheroid growth curves for different values of the necrosis coefficient.

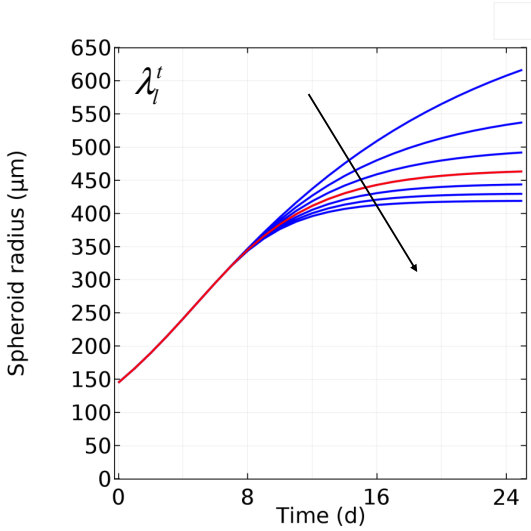

Figure 6: Spheroid growth curves for different values of the lysis coefficient.

Finally, we study the effects of varying α in the mathematical expression for Σ. The value of the derivative Σ' is directly proportional to this parameter, as shown in [12]. We vary the value of α for the ±25, ±50 and ±75% respect to its reference value. Even though we apply a significant variation, there is no apparent effect in the growth curves, which appear superimposed (results not shown). This result may point to the fact that the dynamics of the system, at least for the set of parameters considered, is mainly governed by the constitutive relations for the mass exchange between the phases.

4 CONCLUSIONS

In this work, we have performed a parametric study on a recent mathematical model for tumor spheroid growth [12]. The influence of a set of parameters on the growth curves of the spheroids has been evaluated, and we have provided a discussion of the results. In summary, some of the parameters show a little effect on the growth dynamics, such as the mechanical

coefficient α and the coefficient related to necrosis. On the other hand, other parameters have a significant impact on the final radius reached by the spheroids, namely the critical oxygen level, the coefficient related to growth and the coefficient related to lysis.

This work is certainly open to a number of improvements. In particular, we considered only one nutrient species, namely oxygen, limiting the growth of the tumor mass. Even though the influence of other chemicals is implicitly incorporated in the mass exchange term in (5), the inclusion of different nutrients, growth and necrosis factors could provide additional insights into the evolution of the tumor system [15,16]. Moreover, as it is common in the literature, most of the laws defining the constitutive relations for the mass exchange terms are derived from phenomenological arguments. It is desirable that they could be inferred from experimental measurements and linked to their biochemical and biomechanical background. Finally, here we consider a simple mechanical picture of the tumor tissue, explicitly dependent on the volume fraction of the TCs. Although this assumption describes conveniently the experimental data, it does not take into account several phenomena related to the mechanics of cell interactions at the macroscale, such as their rearrangement after the breakage of the cellular bonds [17].

In the future, we aim to design a new set of experiments that will provide better estimates of the model parameters and help the derivation of the constitutive relations. A better characterization of the interactions between the cancer cells and their microenvironment should offer important insights into the understanding of the disease, and for designing new treatments.

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REFERENCES

[1] Stewart, B. and Wild, C.P., editors. (2014) World Cancer Report 2014.

[2] Longo, D., Fauci, A., Kasper, D. and Hauser, S. (2011) Harrison's principles of internal medicine. McGraw-Hill Professional.

[3] Michor, F., Liphardt, J., Ferrari, M. and Widom, J. (2011) What does physics have to do with cancer? *Nature Reviews Cancer*, Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved. **11**, 657–70. http://dx.doi.org/10.1038/nrc3092

[4] Giverso, C. and Preziosi, L. (2012) Modelling the compression and reorganization of cell aggregates. *Mathematical Medicine and Biology*, **29**, 181–204. http://dx.doi.org/10.1093/imammb/dqr008

[5] Pascal, J., Bearer, E.L., Wang, Z., Koay, E.J., Curley, S. a and Cristini, V. (2013) Mechanistic patient-specific predictive correlation of tumor drug response with microenvironment and perfusion measurements. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 14266–71. http://dx.doi.org/10.1073/pnas.1300619110

[6] Leder, K., Pitter, K., Laplant, Q., Hambardzumyan, D., Ross, B.D., Chan, T.A. et al. (2014) Mathematical modeling of PDGF-driven glioblastoma reveals optimized radiation dosing schedules. *Cell*, **156**, 603–16. http://dx.doi.org/10.1016/j.cell.2013.12.029

[7] Altrock, P.M., Liu, L.L. and Michor, F. (2015) The mathematics of cancer: integrating quantitative models. *Nature Reviews Cancer*, Nature Publishing Group. **15**, 730–45. http://dx.doi.org/10.1038/nrc4029

[8] Sciumè, G., Shelton, S., Gray, W.G., Miller, C.T., Hussain, F., Ferrari, M. et al. (2013) A multiphase model for three-dimensional tumor growth. *New Journal of Physics*, **15**, 015005. http://dx.doi.org/10.1088/1367-2630/15/1/015005

[9] Sciumè, G., Gray, W.G., Hussain, F., Ferrari, M., Decuzzi, P. and Schrefler, B. A. (2013) Three phase flow dynamics in tumor growth. *Computational Mechanics*, **53**, 465–84. http://dx.doi.org/10.1007/s00466-013-0956-2

[10] Sciumè, G., Santagiuliana, R., Ferrari, M., Decuzzi, P. and Schrefler, B.A. (2014) A tumor growth model with deformable ECM. *Physical Biology*, **11**, 65004. http://dx.doi.org/10.1088/1478-3975/11/6/065004

[11] Santagiuliana, R., Stigliano, C., Mascheroni, P., Ferrari, M., Decuzzi, P. and Schrefler, B. a. (2015) The role of cell lysis and matrix deposition in tumor growth modeling. *Advanced Modeling and Simulation in Engineering Sciences*, Springer International Publishing. **2**, 19. http://dx.doi.org/10.1186/s40323-015-0040-x

[12] Mascheroni, P., Stigliano, C., Carfagna, M., Boso, D.P., Preziosi, L., Decuzzi, P. et al. (2016) Predicting the growth of glioblastoma multiforme spheroids using a multiphase porous media model. *Biomechanics and Modeling in Mechanobiology*, Springer Berlin Heidelberg. http://dx.doi.org/10.1007/s10237-015-0755-0

[13] Sutherland, R.M., McCredie, J. a and Inch, W.R. (1971) Growth of multicell spheroids in tissue culture as a model of nodular carcinomas. *Journal of the National Cancer Institute*, **46**, 113–20. http://dx.doi.org/10.1093/jnci/46.1.113

[14] Mikhail, A.S., Eetezadi, S. and Allen, C. (2013) Multicellular tumor spheroids for evaluation of cytotoxicity and tumor growth inhibitory effects of nanomedicines in vitro: A comparison of Docetaxel-loaded block copolymer micelles and Taxotere®. He X, editor. *PLoS ONE*, **8**, e62630. http://dx.doi.org/10.1371/journal.pone.0062630

[15] Chauhan, V.P. and Jain, R.K. (2013) Strategies for advancing cancer nanomedicine. *Nature Materials*, Nature Publishing Group. **12**, 958–62. http://dx.doi.org/10.1038/nmat3792

[16] Jain, R.K., Martin, J.D. and Stylianopoulos, T. (2014) The role of mechanical forces in tumor growth and therapy. *Annual Review of Biomedical Engineering*, **16**, 321–46. http://dx.doi.org/10.1146/annurev-bioeng-071813-105259

[17] Preziosi, L. and Vitale, G. (2011) A multiphase model of tumor and tissue growth including cell adhesion and plastic reorganization. *Mathematical Models and Methods in Applied Sciences*, **21**, 1901–32. http://dx.doi.org/10.1142/S0218202511005593