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Evaluating the influence of mechanical stress on anticancer treatments through a multiphase porous media model / Mascheroni, Pietro; Boso, Daniela; Preziosi, Luigi; Schrefler, Bernhard A.. - In: JOURNAL OF THEORETICAL BIOLOGY. - ISSN 0022-5193. - 421:(2016), pp. 179-188. [10.1016/j.jtbi.2017.03.027]

Availability:

This version is available at: 11583/2670892 since: 2021-03-31T18:52:43Z

Publisher:

Academic Press

Published

DOI:10.1016/j.jtbi.2017.03.027

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<http://dx.doi.org/10.1016/j.jtbi.2017.03.027>

(Article begins on next page)

1 Evaluating the influence of mechanical stress on anticancer treatments through a 2 multiphase porous media model

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8

9 Highlights

- 10 • Low-proliferating cell populations contribute to drug resistance
- 11 • Compressive stresses act on tumors by inhibiting cell proliferation
- 12 • We analyze the combined effects of drug action and mechanical compression
- 13 • Mechanical compression of tumors may compromise drug efficacy

14

15 Abstract

16 Drug resistance is one of the leading causes of poor therapy outcomes in cancer. As several
17 chemotherapeutics are designed to target rapidly dividing cells, the presence of a low-proliferating
18 cell population contributes significantly to treatment resistance. Interestingly, recent studies have
19 shown that compressive stresses acting on tumor spheroids are able to hinder cell proliferation,
20 through a mechanism of growth inhibition. However, studies analyzing the influence of mechanical
21 compression on therapeutic treatment efficacy have still to be performed. In this work, we start
22 from an existing mathematical model for avascular tumors, including the description of mechanical
23 compression. We introduce governing equations for transport and uptake of a chemotherapeutic
24 agent, designed to target cell proliferation. Then, model equations are adapted for tumor spheroids
25 and the combined effect of compressive stresses and drug action is investigated. Interestingly, we
26 find that the variation in tumor spheroid volume, due to the presence of a drug targeting cell
27 proliferation, considerably depends on the compressive stress level of the cell aggregate. Our results
28 suggest that mechanical compression of tumors may compromise the efficacy of chemotherapeutic
29 agents. In particular, a drug dose that is effective in reducing tumor volume for stress-free
30 conditions may not perform equally well in a mechanically compressed environment.

31 **Keywords**

32 Cancer; Chemotherapy; Tumor Spheroids; Mathematical Model; Mechanical Compression

33

34 **1. Introduction**

35 A major hurdle to chemotherapy success is resistance of tumor cells to therapeutic agents. In
36 general, resistance may arise as an intrinsic cellular response or as a result of drug treatment
37 (Zahreddine and Borden, 2013). It is known that the presence of a low-proliferating cell population
38 is one of the leading factors contributing to drug resistance in solid tumors (Mueller-Klieser, 2000;
39 Trédan et al., 2007). In fact, several chemotherapeutic agents are effective against rapidly dividing
40 cells. Moreover, as certain normal tissues display high rates of cellular divisions (such as the gut
41 mucosal and bone marrow cells), there exists a toxicity limit determining the maximum
42 administrable drug dose (Dawidczyk et al., 2014).

43 Such resistance mechanisms, dependent on the proliferative activity of tumor cells, are generally
44 investigated *in vitro* through the use of three-dimensional cell aggregates, known as tumor
45 spheroids (Vinci et al., 2012). Contrary to conventional monolayer cultures, tumor spheroids display
46 heterogeneous cell populations, including quiescent and necrotic cells, together with resistant
47 phenomena to different chemotherapeutic drugs (Mikhail et al., 2013). Cell quiescence results both
48 from the lack of nutrients and growth factors within the tumor, and from adhesion interactions
49 between cells of the same type. Indeed, cells from healthy tissues display a mechanism of “contact
50 inhibition” that regulates proliferation in a crowded environment (Abercrombie and Ambrose,
51 1962). This mechanism allows the cells to stop proliferation as soon as certain cell densities are
52 reached at a given site. Tumor cells exhibit an analogous behavior, even though to a significant lesser
53 extent than their healthy counterpart, and with more relevance in three-dimensional cultures than
54 in monolayers (St Croix et al., 1998).

55 The biochemical pathways underlying contact inhibition are still an active area of research. They are
56 linked to adhesive interactions between neighboring cells, mediated by adhesion proteins such as
57 cadherins. Moreover, these mechanisms include a series of proteins involved in cell cycle regulation.
58 To this regard, the G1 checkpoint, also known as the restriction point (R), represents a fundamental
59 step in the cell cycle, controlling cell commitment to mitosis (Planas-Silva and Weinberg, 1997).
60 Regulation of this cell checkpoint depends on the retinoblastoma protein (pRb). In particular, the

61 hypo-phosphorylated form of pRb prevents progression from the G1 to the S phase of the cell cycle,
62 inhibiting cell duplication. On the other hand, phosphorylation of pRb leads to its inactivation
63 allowing the cell to undergo mitosis. Phosphorylation of pRb depends on cyclin-dependent kinases
64 (cdks), which in turn are subject to the action of cyclins (Dietrich et al., 1997). Finally, the activity of
65 the whole complex is further regulated by several inhibitor proteins, in particular the cyclin-
66 dependent kinase inhibitor p27 (Hengst et al., 1994; Polyak et al., 1994). Interestingly, an over-
67 expression of this protein has been observed following cell-cell contact in three-dimensional
68 cultures, as compared to monolayers (St Croix et al., 1998, 1996; Xing et al., 2005). The adhesive
69 interaction between cells inside tumor spheroids leads to upregulation of p27, which results in cell
70 arrest in a quiescent phase of the cycle. Recently, the expression of p27 has been investigated
71 through a series of experiments involving mechanical compression of three-dimensional cell
72 aggregates (Delarue et al., 2014). Results show that a controlled compressive stress on tumor
73 spheroids inhibits cell proliferation by an over-expression of p27, blocking the cancerous cells at the
74 restriction point of the cell cycle.

75 At the beginning of this introduction, we have observed that the presence of a non-proliferating
76 cellular fraction has important consequences on the therapeutic efficacy of different
77 chemotherapeutic agents. Notably, previous works have shown that a reduction in p27 expression
78 in tumor spheroids could lead to better outcomes in terms of drug performance (St Croix et al.,
79 1998, 1996; Xing et al., 2005). However, experiments quantifying the influence of mechanical stress
80 on drug efficacy have still to be performed. Note that, interestingly, the compressive stresses that
81 can be induced in tumor spheroids are of the same order of magnitude of those measured *in vivo*
82 (Butcher et al., 2009; Fernández-Sánchez et al., 2015; Stylianopoulos et al., 2012), in the range of a
83 few kPa.

84 Phenomena concerning the mechanisms of drug action, as well as the mechanical characterization
85 of the state of a tissue, are difficult to investigate from a pure biological and biochemical framework.
86 To this end, mathematical models provide a valuable tool for establishing which of the biophysical
87 features of the tumor and the stroma are responsible for the observed behaviors. In the last years,
88 several review papers discussing different approaches to cancer modeling have been published
89 (Altrock et al., 2015; Byrne, 2010; Preziosi and Tosin, 2009; Sciumè et al., 2013). Some models
90 describe the action of a therapeutic agent on tumor spheroids (see for example (Frieboes et al.,
91 2009; Goodman et al., 2008; Ward and King, 2003)), whereas others take into account *in vivo*

92 settings, as in (Hossain et al., 2012; Kim et al., 2013; Mpekris et al., 2015). There are also models
93 addressing the effects of mechanical stress on tumor development, such as those in (Kim et al.,
94 2011; Loessner et al., 2013; Stylianopoulos et al., 2012). However, to the authors' knowledge, there
95 is a lack of mathematical models focusing on the interactions between anticancer agents and the
96 mechanical environment surrounding the tumor.

97 The aim of this work is to develop a theoretical framework that is able to take into account these
98 interactions, providing new insights into mechanics-mediated drug resistance. In the following, we
99 specialize our study to tumor spheroids. We address the effects of a chemotherapeutic agent,
100 supposed to target cell proliferation, on these cell aggregates. Then, we evaluate the influence of
101 mechanical compression on treatment efficacy.

102 The remainder of this work is organized as follows. Section 2 describes the mathematical model; the
103 governing equations are presented, together with the assumed constitutive relations and
104 parameter values. In Section 3 we report the results of the model. We start from the effects of
105 different drug concentrations on the spheroid growth curve. Then, we consider a range of
106 mechanical pressures acting on the spheroid surface and investigate their interactions with the
107 treatment. Finally, we test different mathematical expressions for the drug-induced cell death term.
108 Section 4, at the end, presents some concluding remarks.

109

110 **2. Mathematical model**

111 *2.1 Governing equations*

112 We build upon the mathematical model in (Mascheroni et al., 2016) to describe the transport of
113 chemotherapeutic agents within an avascular tumor. The tumor is modeled as a biphasic porous
114 material, and the governing equations are derived from porous media theory. We denote by t the
115 solid phase of the porous medium, constituted by tumor cells (TCs) and ECM. The interstitial fluid
116 (IF) constitutes the fluid phase (ℓ), which permeates the pores of the cellular scaffold. In our
117 description, TCs are divided into living (Lt) and necrotic (Nt) fractions. In addition, we assume that
118 the IF carries a nutrient, namely oxygen (ox), and a drug (ch). We consider a saturated material,
119 where the IF fills all the voids of the porous medium. This results in the saturation constraint:

$$120 \quad \varepsilon^t + \varepsilon^\ell = 1 \quad (1)$$

121 where ε^α denotes the volume fraction of phase α ($\alpha = t, \ell$). The mass balance equations for the
 122 phases in the biphasic system are given by:

$$123 \quad \frac{\partial(\varepsilon^t \rho^t)}{\partial t} + \text{div}(\varepsilon^t \rho^t \mathbf{v}^t) = \overset{\ell \rightarrow t}{M_g} - \overset{t \rightarrow \ell}{M_d} \quad (2)$$

$$124 \quad \frac{\partial(\varepsilon^\ell \rho^\ell)}{\partial t} + \text{div}(\varepsilon^\ell \rho^\ell \mathbf{v}^\ell) = -\overset{\ell \rightarrow t}{M_g} + \overset{t \rightarrow \ell}{M_d} \quad (3)$$

125 where ρ^α is the true mass density and \mathbf{v}^α the velocity of the α phase ($\alpha = t, \ell$). Here $\overset{\ell \rightarrow t}{M_g}$ is the
 126 term responsible for mass exchange between IF and TCs, dependent on cell proliferation; $\overset{t \rightarrow \ell}{M_d}$
 127 represents instead mass exchange between TCs and IF resulting from cell death and their following
 128 degradation. Oxygen and drug are described as species dissolved into the IF, and their mass balance
 129 reads:

$$130 \quad \frac{\partial(\varepsilon^\ell \rho^\ell \omega^{ox})}{\partial t} + \text{div}(\varepsilon^\ell \rho^\ell \omega^{ox} \mathbf{v}^\ell) - \text{div}[\varepsilon^\ell \rho^\ell D^{ox} \text{grad}(\omega^{ox})] = -\overset{ox \rightarrow t}{M_{ox}} \quad (4)$$

$$131 \quad \frac{\partial(\varepsilon^\ell \rho^\ell \omega^{ch})}{\partial t} + \text{div}(\varepsilon^\ell \rho^\ell \omega^{ch} \mathbf{v}^\ell) - \text{div}[\varepsilon^\ell \rho^\ell D^{ch} \text{grad}(\omega^{ch})] = -\overset{ch \rightarrow t}{M_{ch}} \quad (5)$$

132 where ω^β denotes the mass fraction of species β and D^β is its diffusion coefficient ($\beta = ox, ch$).
 133 The terms $\overset{ox \rightarrow t}{M_{ox}}$ and $\overset{ch \rightarrow t}{M_{ch}}$ represent oxygen and drug uptake by TCs, respectively. We describe the
 134 evolution for living and necrotic TCs through the system:

$$135 \quad \frac{\partial(\varepsilon^t \rho^t \omega^{Lt})}{\partial t} + \text{div}(\varepsilon^t \rho^t \omega^{Lt} \mathbf{v}^t) = -\varepsilon^t r^{Nt} + \overset{\ell \rightarrow t}{M_g} \quad (6)$$

$$136 \quad \frac{\partial(\varepsilon^t \rho^t \omega^{Nt})}{\partial t} + \text{div}(\varepsilon^t \rho^t \omega^{Nt} \mathbf{v}^t) = \varepsilon^t r^{Nt} - \overset{t \rightarrow \ell}{M_d} \quad (7)$$

137 where we have denoted by ω^{Lt} and ω^{Nt} the mass fractions of living and necrotic cells, respectively.
 138 Here $\varepsilon^t r^{Nt}$ is an intra-phase mass exchange term, commonly denoted as reaction term, accounting
 139 for the transfer of TCs from living to necrotic. Note that, by summing (6) and (7) we obtain (2)
 140 assuming that:

141
$$\omega^{Lt} = 1 - \omega^{Nt} \quad (8)$$

142

143 Following porous media theory (Lewis and Schrefler, 1998; Pinder and Gray, 2008), the mechanical
 144 stress exerted on the solid phase is described through the effective stress tensor $\mathbf{t}_{\text{eff}}^t$ given by:

145
$$\mathbf{t}_{\text{eff}}^t = \mathbf{t}^t + \alpha_B p^\ell \mathbf{I} \quad (9)$$

146 where \mathbf{I} is the unit tensor, \mathbf{t}^t the total stress tensor, p^ℓ is the fluid pressure in the interstitial fluid
 147 and α_B is Biot's coefficient defined by:

148
$$\alpha_B = 1 - \frac{K}{K_T}, \quad (10)$$

149 with K bulk modulus of the unsaturated skeleton and K_T bulk modulus of the solid phase. Then,
 150 we can state the linear momentum balance law for the tissue as (Lewis and Schrefler, 1998):

151
$$\text{div} \mathbf{t}^t = \text{div} (\mathbf{t}_{\text{eff}}^t - \alpha_B p^\ell \mathbf{I}) = 0 \quad (11)$$

152 Note that in (9) the tensile components of the stress tensors \mathbf{t}^t and $\mathbf{t}_{\text{eff}}^t$ are assumed positive.

153

154 2.2 Constitutive relations

155 In (Mascheroni et al., 2016), constitutive relationships for the effective stress and the mass transfer
 156 terms have been formulated. In particular, we have assumed the following form for the effective
 157 stress:

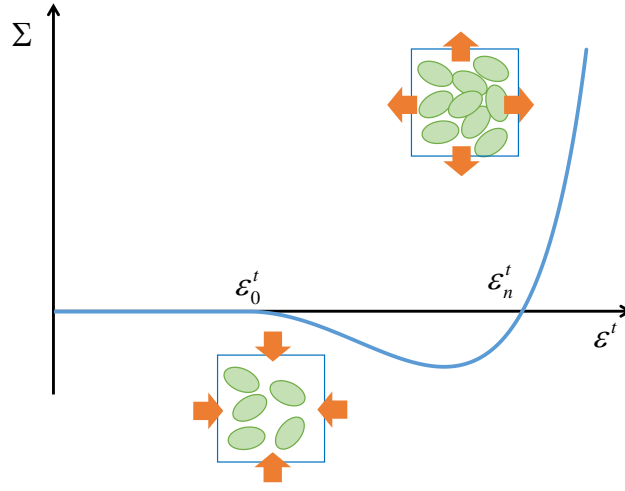
158
$$\mathbf{t}_{\text{eff}}^t = -\Sigma(\varepsilon^t) \mathbf{I} \quad (12)$$

159 with $\Sigma(\varepsilon^t)$ given by:

160
$$\Sigma(\varepsilon^t) = \begin{cases} \alpha (\varepsilon^t - \varepsilon_0^t)^2 \left[\frac{1 - \varepsilon_n^t}{(1 - \varepsilon^t)^\beta} - \frac{1}{(1 - \varepsilon^t)^{\beta-1}} \right], & \text{if } \varepsilon^t > \varepsilon_0^t \\ 0, & \text{otherwise} \end{cases} \quad (13)$$

161 This pseudo-potential law describes cells that do not interact if their volume fraction is below a given
 162 threshold (ε_0^t). Otherwise, they start to interact and develop attraction forces as long as their
 163 volume fraction is below a control value (ε_n^t). Finally, if TCs become too densely packed exhibiting a
 164 high volume fraction, they start to repel each other. This behavior is schematized in Figure 1. In the
 165 following we will denote by $\Sigma'(\varepsilon^t)$ the derivative of $\Sigma(\varepsilon^t)$ with respect to ε^t .

166



167

168 **Figure 1** Schematic for the stress function $\Sigma(\varepsilon^t)$, depicting the behavior of cells at different volume fractions.

169

170 The mass exchange terms in equation (2) represent TC growth and death, respectively. The first
 171 term describes cell proliferation and depends on the transfer of mass between the IF and the living
 172 fraction of the tumor. It takes the form:

173

$$174 \quad M_g^{\ell \rightarrow t} = \gamma_g^t \left\langle \frac{\omega^{ox} - \omega_{crit}^{ox}}{\omega_{env}^{ox} - \omega_{crit}^{ox}} \right\rangle_+ \left(1 - \delta_1 \frac{\langle \Sigma \rangle_+}{\langle \Sigma \rangle_+ + \delta_2} \right) \omega^{Lt} \varepsilon^t \quad (14)$$

175 here the coefficient γ_g^t accounts for oxygen uptake and for mass of IF that becomes tumor due to
 176 cell growth; ω_{crit}^{ox} is the critical mass fraction of oxygen, below which growth is inhibited, and ω_{env}^{ox} is
 177 the reference mass fraction of oxygen in the environment. The Macaulay brackets $\langle \cdot \rangle_+$ indicate the
 178 positive value of their argument: since the oxygen mass fraction ω^{ox} within the tumor can only be

179 equal to or smaller than ω_{env}^{ox} , the brackets will return a number between one ($\omega^{ox} = \omega_{env}^{ox}$) and zero
 180 ($\omega^{ox} < \omega_{env}^{ox}$). The term in round squares describes growth inhibition by mechanical stress. The
 181 constants δ_1 and δ_2 (with $\delta_1 < 1$) regulate the action of mechanical stress on cell proliferation and,
 182 together with the term $\langle \Sigma \rangle_+$, model the inhibitory effect of compression on tumor cells proliferation
 183 (Cheng et al., 2009; Helmlinger et al., 1997; Montel et al., 2012).

184 The rate of TC death in equation (2) is given by:

$$185 \quad M_{d,ly}^{t \rightarrow \ell} = M_{d,ly}^{t \rightarrow \ell} + M_{d,ch}^{t \rightarrow \ell} \quad (15)$$

186 where the two contributions are related to cell lysis and drug action. In particular, the first term is
 187 given by:

$$188 \quad M_{d,ly}^{t \rightarrow \ell} = \lambda_{\ell}^t \omega^{Nt} \varepsilon^t \quad (16)$$

189 where the coefficient λ_{ℓ}^t takes into account cellular degradation and mass conversion of necrotic
 190 cells into IF. The second term takes the form:

$$191 \quad M_{d,ch}^{t \rightarrow \ell} = f_{ch} \lambda_{ch}^t \omega^{ch} \omega^{Lt} \varepsilon^t \quad (17)$$

192 Here λ_{ch}^t accounts for the rate of drug-induced cell death. The function f_{ch} is related to the
 193 mechanism of action of the drug that is considered. Since we are interested in drugs that target TC
 194 proliferation, we assume f_{ch} to depend on the growth term in (14):

$$195 \quad f_{ch}(\omega^{ox}, \Sigma) = \frac{M_g^{\ell \rightarrow t}}{\max(M_g^{\ell \rightarrow t})} = \left\langle \frac{\omega^{ox} - \omega_{crit}^{ox}}{\omega_{env}^{ox} - \omega_{crit}^{ox}} \right\rangle_+ \left(1 - \delta_1 \frac{\langle \Sigma \rangle_+}{\langle \Sigma \rangle_+ + \delta_2} \right) \quad (18)$$

196 where we highlight the dependence of f_{ch} on both the nutrient mass fraction ω^{ox} and the
 197 mechanical stress Σ . In this way, the drug is most effective on the TCs that are well nourished and
 198 not compressed. Note that, depending on the particular drug that is considered, different choices
 199 for f_{ch} are possible (for example, in this framework it is possible to simulate drugs targeting hypoxia
 200 or specific cellular species in the tumor).

201 The rate of necrosis of living tumor cells in equation (6) is described by:

202

$$\varepsilon^t \Gamma^{Nt} = \gamma_n^t \left\langle \frac{\omega_{crit}^{ox} - \omega^{ox}}{\omega_{env}^{ox} - \omega_{crit}^{ox}} \right\rangle_+ \omega^{Lt} \varepsilon^t \quad (19)$$

203

where the parameter γ_n^t regulates the rate of cell necrosis. The terms in the Macaulay brackets

204

represent cell death by lack of nutrients.

205

During growth, TCs consume nutrients from the IF, a process that is described by the mass exchange

206

term in equation (4):

207

$$\frac{M_{ox}^{ox \rightarrow t}}{M_{ox}} = \gamma_0^t \frac{\omega^{ox}}{\omega^{ox} + c^{ox}} \omega^{Lt} \varepsilon^t \quad (20)$$

208

This expression accounts for the dependence of oxygen consumption on its local level in the tumor.

209

The coefficients γ_0^t and c^{ox} represent the order of magnitude of oxygen uptake and the oxygen

210

mass fraction at which consumption is reduced by half, respectively.

211

Finally, the mass transfer term related to drug uptake in equation (5) takes the form:

212

$$\frac{M_{ch}^{ch \rightarrow t}}{M_{ch}} = \gamma_{ch}^t \omega^{ch} \omega^{Lt} \varepsilon^t \quad (21)$$

213

where we assumed the simplest kinetics for drug uptake (i.e. linear), with γ_{ch}^t accounting for the

214

drug uptake rate by living TCs (Frieboes et al., 2009; Weinberg et al., 2007).

215

216

2.3 Model specialization to tumor spheroids

217

The equations of the model can be specialized to the case of tumor spheroids, following a similar

218

procedure to that in (Mascheroni et al., 2016). The resulting system for the TC volume fraction,

219

necrotic mass fraction, and oxygen and drug mass fractions can be summarized as:

220

$$\frac{\partial \varepsilon^t}{\partial t} - \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \varepsilon^t \frac{k}{\mu^\ell} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{\rho} \left(\overset{\ell \rightarrow t}{M}_g - \overset{t \rightarrow \ell}{M}_d \right) = 0 \quad (22)$$

221

$$\frac{\partial (\omega^{Nt} \varepsilon^t)}{\partial t} - \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \varepsilon^t \omega^{Nt} \frac{k}{\mu^\ell} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{\rho} \left(\varepsilon^t r^{Nt} - \overset{t \rightarrow \ell}{M}_{d.ly} \right) = 0 \quad (23)$$

222

$$\frac{\partial [(1 - \varepsilon^t) \omega^{ox}]}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \varepsilon^t \omega^{ox} \frac{k}{\mu^\ell} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 (1 - \varepsilon^t) D^{ox} \frac{\partial \omega^{ox}}{\partial r} \right] + \frac{1}{\rho} \overset{ox \rightarrow t}{M}_{ox} = 0 \quad (24)$$

$$223 \quad \frac{\partial \left[(1 - \varepsilon^t) \omega^{ch} \right]}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \varepsilon^t \omega^{ch} \frac{k}{\mu^\ell} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \right) - \frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 (1 - \varepsilon^t) D^{ch} \frac{\partial \omega^{ch}}{\partial r} \right] + \frac{1}{\rho} \overset{ch \rightarrow t}{M} = 0 \quad (25)$$

224 We have adopted spherical symmetry, and r is the radial coordinate over the spheroid radius. The
 225 parameters k and μ^ℓ are the intrinsic permeability of the cellular scaffold and the dynamic viscosity
 226 of IF, respectively. They arise by assuming Darcy's law for the relative velocity of the two phases
 227 (Mascheroni et al., 2016; Sciumè et al., 2013). Moreover, we take the phases to be incompressible
 228 and assign a common value for their densities, which we denote by the constant ρ . Note that this
 229 leads to $\alpha_B = 1$. Then, we model the growth of the spheroid as a free-boundary problem, where the
 230 interface constituted by TCs is a material surface for the TCs that moves with velocity v^t , given by:

$$231 \quad \frac{dR}{dt} = v^t = - \frac{k}{\mu^\ell} \Sigma' \frac{\partial \varepsilon^t}{\partial r} \Big|_{r=R} \quad (26)$$

232 where R is the external radius of the spheroid. The closed form of the differential problem is then
 233 obtained by defining a proper set of boundary and initial conditions. In particular, regularity at the
 234 spheroid center requires:

$$235 \quad \frac{\partial \varepsilon^t}{\partial r} = \frac{\partial \omega^{Nt}}{\partial r} = \frac{\partial \omega^{ox}}{\partial r} = \frac{\partial \omega^{ch}}{\partial r} = 0, \quad \text{in } r = 0, \quad (27)$$

236 while we enforce Dirichlet boundary conditions on the tumor external surface:

$$237 \quad \varepsilon^t = \varepsilon_{ext}^t, \quad \omega^{Nt} = 0, \quad \omega^{ox} = \omega_{env}^{ox}, \quad \omega^{ch} = \omega_{env}^{ch}, \quad \text{in } r = R. \quad (28)$$

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

$$239 \quad \varepsilon^t = \varepsilon_{ext}^t, \quad \omega^{Nt} = 0, \quad \omega^{ox} = \omega_{env}^{ox}, \quad \omega^{ch} = 0, \quad \text{on } 0 < r < R \text{ at } t = 0. \quad (29)$$

240

241 2.4 Model parameters

242 The parameters used in the model are listed in Table 1. Some of the values are taken from
 243 (Mascheroni et al., 2016), where the model results are compared to experimental data. In this work,
 244 we need to add the values for the parameters appearing in the equations governing drug transport
 245 and uptake. For these quantities we assume the values in (Frieboes et al., 2009), obtained for
 246 spheroids treated with Doxorubicin. Actually, the parameter governing drug-induced cell death,

247 λ_{ch}^t , depends on the particular therapeutic agent and cell line that are considered. Here it is selected
 248 to produce a reasonable response of the model when the spheroids are subjected to the given drug
 249 concentrations. Note that, as it will be shown in Section 3.3, model results will not be significantly
 250 affected by this choice.

251

252 **Table 1** Parameters used in the model.

Parameter	Value	Unit	Reference
ω_{env}^{ox}	7.7×10^{-6}	(-)	(Mueller-Klieser et al., 1986; Mueller-Klieser and Sutherland, 1982)
c^{ox}	1.48×10^{-7}	(-)	(Casciari et al., 1992a, 1992b)
γ_0^t	3.0×10^{-4}	kg/(m ³ · s)	(Casciari et al., 1992a, 1992b)
β	0.5	(-)	(Byrne and Preziosi, 2003)
ε_n^t	0.8	(-)	(Byrne and Preziosi, 2003)
ε_0^t	1/3	(-)	(Byrne and Preziosi, 2003)
k	1.8×10^{-15}	m ²	(Netti et al., 2000)
μ^ℓ	1.0×10^{-3}	Pa · s	(Sciumè et al., 2013b)
D^{ox}	3.2×10^{-9}	m ² /s	(Sciumè et al., 2013b)
ρ	1.0×10^3	kg/m ³	(Sciumè et al., 2013b)
ω_{crit}^{ox}	2.0×10^{-6}	(-)	(Mascheroni et al., 2016)
γ_g^t	5.4×10^{-3}	kg/(m ³ · s)	(Mascheroni et al., 2016)
γ_n^t	1.5×10^{-1}	kg/(m ³ · s)	(Mascheroni et al., 2016)
λ_ℓ^t	1.15×10^{-2}	kg/(m ³ · s)	(Mascheroni et al., 2016)
α	1.0×10^5	Pa	(Mascheroni et al., 2016)
ω_{env}^{ch}	$8.696 \div 271.76 \times 10^{-9}$	(-)	(Frieboes et al., 2009)
D^{ch}	9.375×10^{-14}	m ² /s	(Frieboes et al., 2009)
γ_{ch}^t	1.157×10^{-2}	kg/(m ³ · s)	(Frieboes et al., 2009)
λ_{ch}^t	5.0×10^4	kg/(m ³ · s)	(-)

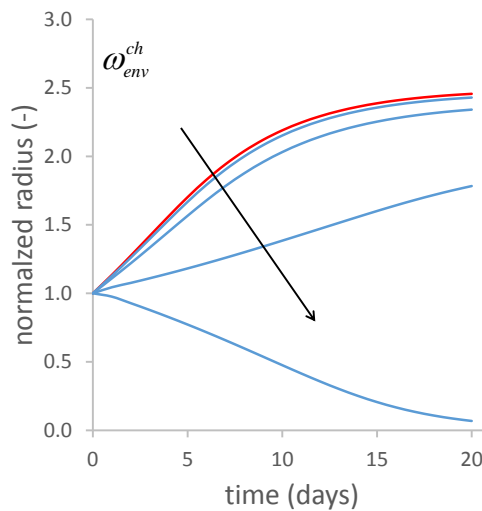
253

254

255 **3. Results**

256 *3.1 Tumor spheroid growth in the presence of a drug*

257 In this section we test the effects of a drug that targets cell proliferation in a three-dimensional cell
258 aggregate. We consider first tumor spheroids that grow suspended in culture medium, subject to
259 different drug concentrations. We assume drug concentration at spheroid boundary to start from
260 zero and, following a ramp, to reach the final value ω_{env}^{ch} after 3h.



261

262 **Figure 2** Effect of different drug concentrations on spheroid growth. The red line refers to a spheroid grown in the
263 absence of drug. The other lines are for $\omega_{env}^{ch} = 0.086, 0.347, 1.391, 2.717 \times 10^{-7}$.

264

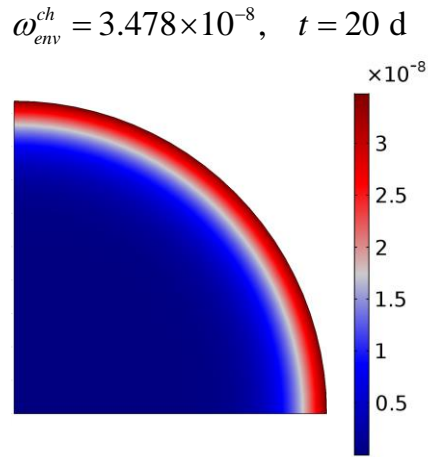
265 In Figure 2, we show the evolution of the spheroid radius over time for different drug mass fractions
266 (i.e. $\omega_{env}^{ch} = 0.086, 0.347, 1.391, 2.717 \times 10^{-7}$). Here, the arrow points in the direction of increasing
267 ω_{env}^{ch} . We consider the normalized value of the spheroid radius, namely the ratio between the
268 present value of the radius and the initial radius of the spheroid (200 μm in this case). The red line
269 represents a spheroid grown in the absence of drug. We can distinguish between the first stages of
270 growth, displaying an exponential/linear behavior, followed by a phase of growth saturation where
271 the radius tends to a steady value. Low concentrations of drug do not alter the shape of the growth
272 curve, whereas for high levels of the chemotherapeutic agent the spheroid starts to shrink and, for
273 the highest value of ω_{env}^{ch} , growth is almost completely inhibited. This behavior closely resembles
274 the growth curves obtained for example in (Kim et al., 2010; Mikhail et al., 2013), where spheroids
275 from various cell lines are subject to different drugs.

276 Figure 3 shows the drug mass fraction inside the spheroid for an intermediate value of ω_{env}^{ch} , at the
277 end of the simulation. Note the steep gradient of drug appearing from the boundary towards the
278 center of the cell aggregate. In this case, the therapeutic agent can exert its effect only over the
279 outermost region of the spheroid. This phenomenon arises as a consequence of poor diffusion of
280 the drug molecules inside the spheroid and due to drug uptake by proliferating TCs. Interestingly,
281 similar results are obtained in the experimental literature (see for example (Gong et al., 2015; Wang
282 et al., 2013)), analyzing the penetration of free drug into a spheroid.

283 Then, we look for the value of drug mass fraction that is able to provide a reduction of 50% in
284 spheroid volume (usually identified with the label IC_{50} , for “half maximal inhibitory concentration”
285 (Curtis et al., 2016)). We find a value of $\omega_{env}^{ch} = 1.185 \times 10^{-7}$, which we will denote from now on with
286 IC_{50} . The growth curve relative to this drug mass fraction is shown in Figure 4.a, where we report
287 the evolution of the normalized volume (i.e. the ratio between the spheroid volume and its initial
288 volume) over time. The evolution of oxygen mass fraction over the spheroid radius is represented
289 in Figure 4.b. Note the steep oxygen gradients at later times of the simulation, from the spheroid
290 boundary towards its interior. The necrotic mass fraction of TCs is displayed in Figure 4.c. A necrotic
291 population appears after a few days from the beginning of the simulation and gives rise to a necrotic
292 core at later days. Both Figures 4.b and 4.c refer to a spheroid not treated with the drug, whereas
293 the second row of Figures (4.d-f) pertains to a spheroid grown in the presence of a drug with a mass
294 fraction equal to IC_{50} . The drug mass fraction over the spheroid radius is presented in Figure 4.d.
295 Note that, after a few days from the beginning of the simulation, the therapeutic agent is mainly
296 distributed over the spheroid periphery. Figure 4.e shows the oxygen mass fraction in the drug-
297 treated spheroid. We can observe a behavior similar to the one in Figure 4.b, but this time over a
298 smaller spheroid. Finally, the necrotic mass fraction in a spheroid subjected to the drug is shown in
299 Figure 4.f. Compared to Figure 4.c, here the necrotic core is less extended and appears at later times
300 in the simulation. This may be due to a smaller mass fraction of LTCs that can undergo necrosis,
301 deriving from LTC killing by the chemotherapeutic agent.

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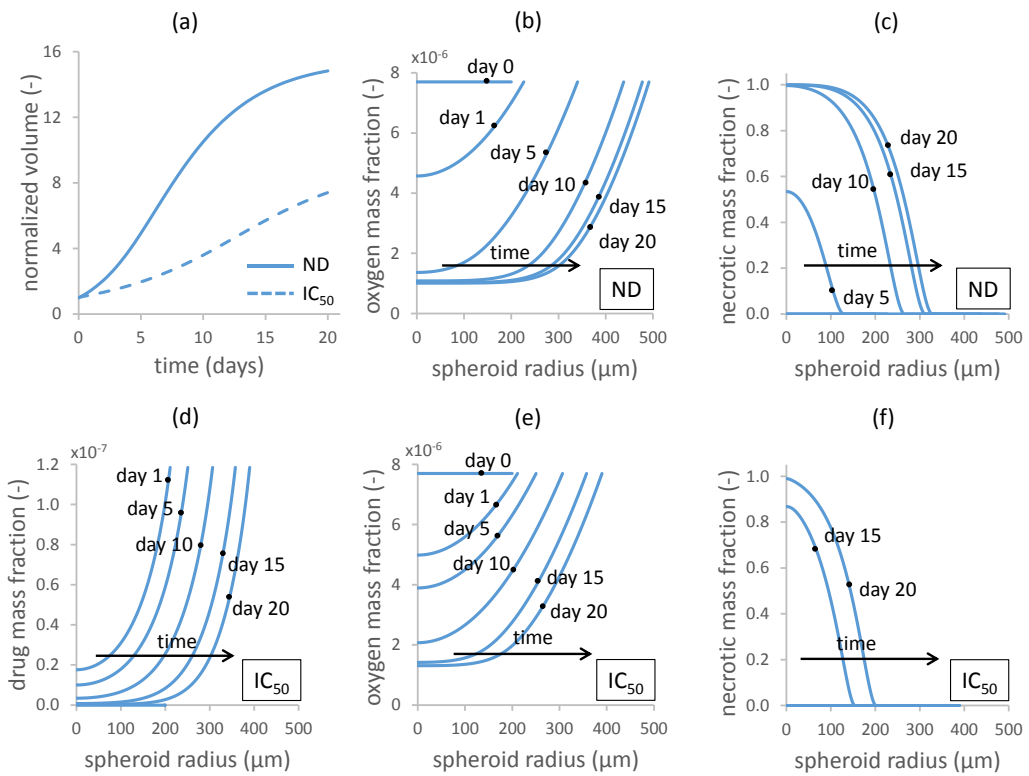
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Figure 3 Drug mass fraction inside the spheroid at day 20 and for $\omega_{env}^{ch} = 3.478 \times 10^{-8}$.

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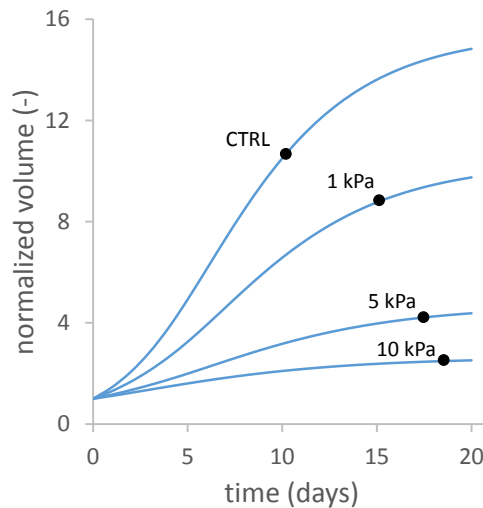
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Figure 4 Comparison between a spheroid grown without an external drug (ND) and one treated with a drug mass fraction equal to IC_{50} .

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Figure 5 Normalized volumes of spheroids grown under different external mechanical pressures.

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316 3.2 Effect of mechanical compression on drug efficacy

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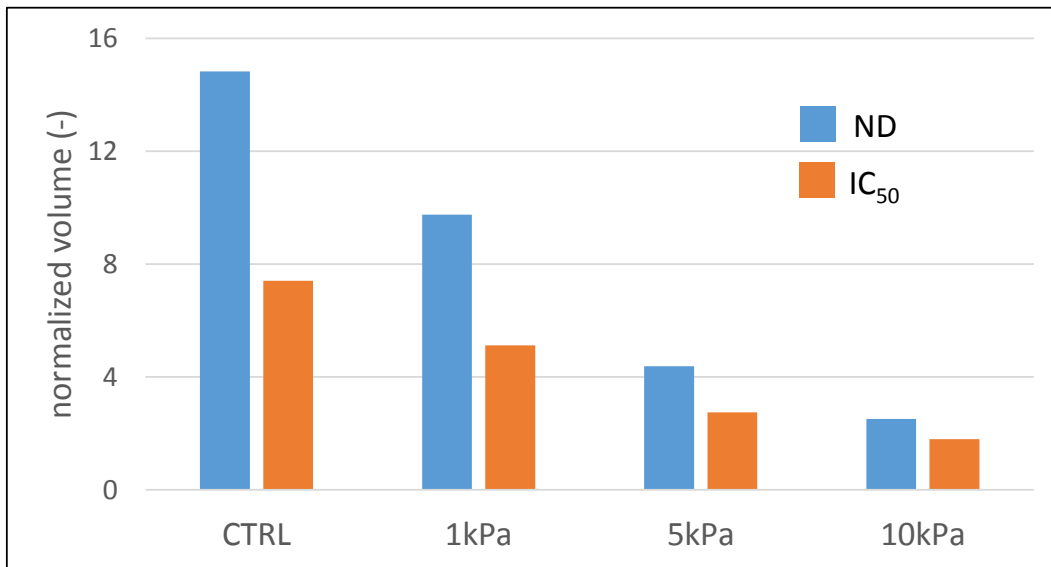
In (Mascheroni et al., 2016), we investigated the effects of an external mechanical pressure on the growth curves of tumor spheroids. Figure 5 report these previous findings, in terms of the evolution of the normalized volumes of spheroids subjected to different compressive stresses. We consider four compression levels, ranging from 1kPa to 10kPa. The growth of the most compressed spheroid shows a 7-fold reduction when compared to the control case (grown in the absence of an external stress). Note that the inhibitory effect of compressive stresses is included in the equations through the constitutive relation in (14). We make use of these results to test our newly introduced framework for drug transport and uptake in the spheroid. In particular, we apply the same external mass fraction of drug (IC_{50}) to each of the compression tests. Then, we check for variations in spheroid volumes with respect to the case with no drug added to the culture medium (Figure 6 and 7). Figure 6 compares the normalized volumes of spheroids undergoing different compressive stresses. We test spheroids in the absence (ND) or presence (IC_{50}) of a chemotherapeutic drug. Both the series, ND and IC_{50} , exhibit the same decreasing trend, although with a slower volume reduction for drug-treated spheroids. The variation between the two volumes for each compressive condition is shown in Figure 7. According to the definition of IC_{50} , the control case displays a 50% reduction in volume. Interestingly, the series exhibit a percentage variation decreasing with the extent of mechanical compression, as highlighted by the black arrow. The case undergoing maximum compression shows a reduction of about 30% in volume reduction. The observed behavior arises as a consequence of a lower proliferation index within the spheroid. In fact, mechanical stress inhibits

336 cell proliferation via equation (14) of the model, providing smaller values for the growth term as
337 compression increases. Since our discussion is based on drugs that target cell duplication, growth
338 inhibition is responsible for a cell population over which the therapeutic agent is less effective. Note
339 that this effect could be relevant for *in vivo* applications: a drug concentration that is known to be
340 effective in a particular regime (such as 3D cultures) could not provide the same results when the
341 tumor is subjected to mechanical compression. Moreover, since several drug screenings are
342 evaluated on monolayer cultures, such effects arising from a full three-dimensional setting may be
343 overlooked (Friedrich et al., 2009).

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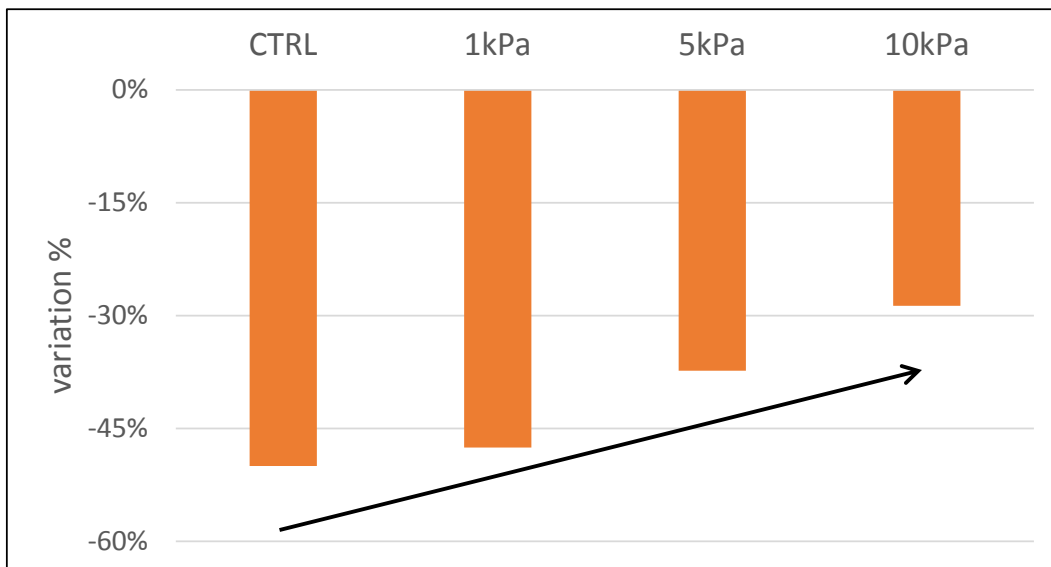
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348 **Figure 6** Comparison of the normalized volumes of spheroids subject to different mechanical stress, grown in the
349 absence of drug (ND) or subject to a drug concentration of IC₅₀.

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354 **Figure 7** Variation in spheroid volume due to the action of a chemotherapeutic drug at different external mechanical
355 pressures. As shown by the arrow, the efficacy of proliferation targeting drugs is less effective for higher tumor
356 compressions, because of stress inhibition of growth.

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358 *3.3 Response of the model for different forms of the drug-induced death term*

359 To confirm that model results are not biased by the particular choice of the term in (17), we test
360 different mathematical expressions accounting for drug-induced cell death. The simplest
361 hypothesis, assumed in (17), considers cell death to be proportional to the local amount of drug. In
362 the following, we will refer to this case as the “linear” one. We introduce two additional
363 relationships, given by:

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$$M_{d, ch}^{t \rightarrow \ell} = f_{ch} \frac{m_1 \omega^{ch}}{\omega^{ch} + m_2} \omega^{L_t} \varepsilon^t \quad (30)$$

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$$M_{d, ch}^{t \rightarrow \ell} = f_{ch} P_1 (\omega^{ch})^{P_2} \omega^{L_t} \varepsilon^t \quad (31)$$

366 In (30), we assume a dependence of the Michaelis-Menten type; in (31) the assumed relationship
367 takes the form of a power law. Note that, as the functional dependence on the local drug
368 concentration changes, these relations give rise to new values for the inhibitory concentration IC_{50} .
369 We report the new IC_{50} and the values for the parameters that characterize the above expressions
370 in Table 2. Once the new forms for the drug-induced cell death term are implemented into the
371 model, we perform the same numerical tests of the previous section to analyze the coupled effect
372 of drug action and mechanical compression. In Figure 8, we report the variation in terms of spheroid
373 volume induced by the drug for different compressive stresses. Like in the previous Figure, the first
374 series of data serves as a control and indicates a variation of 50% with respect to the drug-free
375 condition. The other series are related to the different compression regimes and compare the model
376 response for the different mathematical relationships assumed for the death term. It is possible to
377 observe that the variation in volume reduction is similar to the linear case, analyzed in the previous
378 section. The effect of mechanical compression on drug efficacy described previously does not seem
379 therefore to be originated from the particular mathematical form adopted for the death term.

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384 **Table 2** Parameter values for the relations assumed in the cell death term.

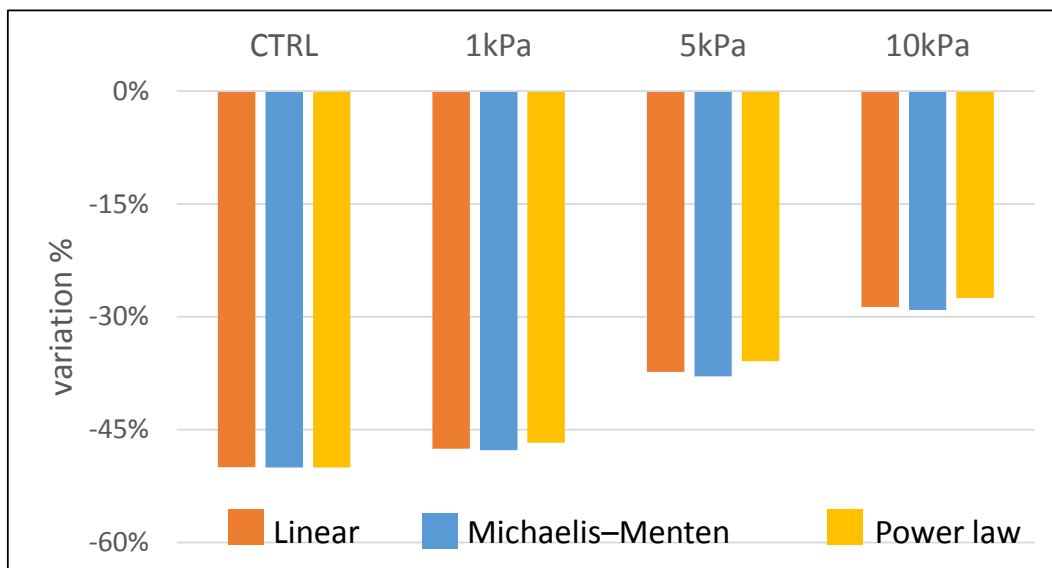
Relation	Parameter	Value	Unit	IC ₅₀
Linear	λ'_{ch}	5.0×10^4	kg / (m ³ · s)	1.185×10^{-7}
Michaelis-Menten	m_1	1.5×10^{-2}	kg / (m ³ · s)	5.345×10^{-8}
	m_2	1.0×10^{-7}	(-)	
Power law	p_1	2.5×10^{11}	kg / (m ³ · s)	1.862×10^{-7}
	p_2	2	(-)	

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390 **Figure 8** Effect of different mathematical relations on spheroid volume variation.

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394 **4. Conclusion**

395 In this work, we introduce equations for drug transport and uptake by TCs in our previous
396 mathematical model for avascular tumor growth. Then, we adapt the equations for the tumor
397 spheroid case and test the effects of a proliferation targeting drug on spheroid growth curves. We
398 observe a qualitative agreement between model results and experimental literature (Gong et al.,
399 2015; Kim et al., 2010; Mikhail et al., 2013; Wang et al., 2013). Then, we simulate tumor spheroids
400 undergoing mechanical compressive stresses of different amplitudes and consider their volume
401 reduction due to the presence of a therapeutic agent. Interestingly, we notice a decreased growth
402 inhibition efficacy of the drug in terms of the final volumes reached by the spheroids, arising because
403 of compressive stresses. Finally, we test three different mathematical expressions for the cell death
404 term induced by the therapeutic agent. The resulting predictions are similar for all the tested
405 relations, suggesting that the particular form of the adopted constitutive relation does not influence
406 model response. Taken together, these results suggest that mechanical compression of tumor
407 spheroids may compromise the efficacy of a chemotherapeutic agent targeting cell proliferation.

408 As several simplifying assumptions are considered in the work, the model is certainly open to further
409 improvements. In particular, here we model only one nutrient species, i.e. oxygen, diffusing in the
410 interstitial fluid and regulating TC proliferation. Even though the presence of other chemicals is
411 implicitly contained within the mass exchange term in (14), future inclusion of additional nutrients,
412 growth and necrosis factors could provide a more detailed description of the tumor system
413 (Chauhan and Jain, 2013; Jain et al., 2014). Moreover, since the particular physicochemical
414 environment in which the tumor is embedded affects significantly the outcomes of therapies (see
415 for example (Luk et al., 1990), (Seebacher et al., 2015)), proper consideration of these factors would
416 result in a better description of drug dynamics. Notably, some experiments couple therapeutic
417 agents to nanoparticle formulations, enabling a larger penetration into the tumor (Kim et al., 2010;
418 Wang et al., 2013). Note that this latter kind of results can be integrated in the current model, once
419 suitable mechanisms for nanoparticle delivery are hypothesized. Another point requiring some
420 attention is the proper choice of constitutive relations. As it happens frequently in literature, most
421 of these laws are derived from phenomenological arguments. More experimental work is needed
422 to link the mathematical form assigned to the various terms to the underlying biology. This kind of
423 reasoning should be applied to the constitutive relations accounting for the drug uptake and the
424 following effects on TCs, as well as the mechanical description of the tumor ensemble. For the latter,

425 here we consider a simple law, linking the stress in the tissue to the local volume fraction of tumor
426 cells. This assumption provides a great simplification of the equations and is shown to give a good
427 description of experimental observations (Mascheroni et al., 2016). However, it neglects several
428 phenomena related to the mechanical behavior of a biological tissue. For example, viscoplastic
429 effects existing at smaller timescales than those of cell proliferation are not taken into account
430 (Forgacs et al., 1998; Givero and Preziosi, 2012). Also, breaking and formation of cellular bonds
431 during tumor development should be included to give a more complete description (Ambrosi et al.,
432 2012; Preziosi et al., 2010). Finally, we highlight the need for experiments addressing the
433 interactions between therapeutic agents and tumor mechanical environment. These experiments
434 will serve to calibrate the parameters in the equations and to test model results. Part of future
435 experimental work should also be devoted to the biochemical understanding of the growth
436 inhibition process following mechanical stress. Although some work is already present in the
437 literature (Cheng et al., 2009; Delarue et al., 2014; Loessner et al., 2013), several details remain to
438 be elucidated. New investigations analyzing the interactions between the tumor and its bio-
439 mechanical environment should allow for a better understanding of disease progression, with the
440 final goal of aiding the design of effective therapeutic treatments.

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442 **Acknowledgements**

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445 **References**

- 446 Abercrombie, M., Ambrose, E.J., 1962. The surface properties of cancer cells: a review. *Cancer Res.* 22, 525–48.
- 447 Altrock, P.M., Liu, L.L., Michor, F., 2015. The mathematics of cancer: integrating quantitative models. *Nat. Rev. Cancer* 15, 730–745.
448 doi:10.1038/nrc4029
- 449 Ambrosi, D., Preziosi, L., Vitale, G., 2012. The interplay between stress and growth in solid tumors. *Mech. Res. Commun.* 42, 87–91.
450 doi:10.1016/j.mechrescom.2012.01.002
- 451 Butcher, D.T., Alliston, T., Weaver, V.M., 2009. A tense situation: forcing tumour progression. *Nat. Rev. Cancer* 9, 108–122.
452 doi:10.1038/nrc2544
- 453 Byrne, H., Preziosi, L., 2003. Modelling solid tumour growth using the theory of mixtures. *Math. Med. Biol.* 20, 341–66.
454 doi:10.1093/imammb/20.4.341
- 455 Byrne, H.M., 2010. Dissecting cancer through mathematics: from the cell to the animal model. *Nat. Rev. Cancer* 10, 221–30.
456 doi:10.1038/nrc2808
- 457 Casciari, J.J., Sotirchos, S. V., Sutherland, R.M., 1992a. Mathematical modelling of microenvironment and growth in EMT6/Ro
458 multicellular tumour spheroids. *Cell Prolif.* 25, 1–22. doi:10.1111/j.1365-2184.1992.tb01433.x
- 459 Casciari, J.J., Sotirchos, S. V., Sutherland, R.M., 1992b. Variations in tumor cell growth rates and metabolism with oxygen
460 concentration, glucose concentration, and extracellular pH. *J. Cell. Physiol.* 151, 386–94. doi:10.1002/jcp.1041510220
- 461 Chauhan, V.P., Jain, R.K., 2013. Strategies for advancing cancer nanomedicine. *Nat. Mater.* 12, 958–62. doi:10.1038/nmat3792

- 462 Cheng, G., Tse, J., Jain, R.K., Munn, L.L., 2009. Micro-environmental mechanical stress controls tumor spheroid size and morphology
463 by suppressing proliferation and inducing apoptosis in cancer cells. *PLoS One* 4, e4632. doi:10.1371/journal.pone.0004632
- 464 Curtis, L.T., England, C.G., Wu, M., Lowengrub, J., Frieboes, H.B., 2016. An interdisciplinary computational/experimental approach
465 to evaluate drug-loaded gold nanoparticle tumor cytotoxicity. *Nanomedicine* 11, 197–216. doi:10.2217/nnm.15.195
- 466 Dawidczyk, C.M., Kim, C., Park, J.H., Russell, L.M., Lee, K.H., Pomper, M.G., Searson, P.C., 2014. State-of-the-art in design rules for
467 drug delivery platforms: lessons learned from FDA-approved nanomedicines. *J. Control. Release* 187, 133–44.
468 doi:10.1016/j.jconrel.2014.05.036
- 469 Delarue, M., Montel, F., Vignjevic, D., Prost, J., Joanny, J.-F., Cappello, G., 2014. Compressive stress inhibits proliferation in tumor
470 spheroids through a volume limitation. *Biophys. J.* 107, 1821–8. doi:10.1016/j.bpj.2014.08.031
- 471 Dietrich, C., Wallenfang, K., Oesch, F., Wieser, R., 1997. Differences in the mechanisms of growth control in contact-inhibited and
472 serum-deprived human fibroblasts. *Oncogene* 15, 2743–7. doi:10.1038/sj.onc.1201439
- 473 Fernández-Sánchez, M.E., Barbier, S., Whitehead, J., Béalle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperon, A.,
474 Brullé, L., Girard, E., Servant, N., Rio-Frio, T., Marie, H., Lesieur, S., Housset, C., Gennisson, J.-L., Tanter, M., Ménager, C., Fre,
475 S., Robine, S., Farge, E., 2015. Mechanical induction of the tumorigenic β -catenin pathway by tumour growth pressure.
476 *Nature* 523, 92–95. doi:10.1038/nature14329
- 477 Forgacs, G., Foty, R. a, Shafir, Y., Steinberg, M.S., 1998. Viscoelastic properties of living embryonic tissues: a quantitative study.
478 *Biophys. J.* 74, 2227–34. doi:10.1016/S0006-3495(98)77932-9
- 479 Frieboes, H.B., Edgerton, M.E., Fruehauf, J.P., Rose, F.R.A.J., Worrall, L.K., Gatenby, R. a, Ferrari, M., Cristini, V., 2009. Prediction of
480 drug response in breast cancer using integrative experimental/computational modeling. *Cancer Res.* 69, 4484–92.
481 doi:10.1158/0008-5472.CAN-08-3740
- 482 Friedrich, J., Seidel, C., Ebner, R., Kunz-Schughart, L.A., 2009. Spheroid-based drug screen: considerations and practical approach.
483 *Nat. Protoc.* 4, 309–324. doi:10.1038/nprot.2008.226
- 484 Givero, C., Preziosi, L., 2012. Modelling the compression and reorganization of cell aggregates. *Math. Med. Biol.* 29, 181–204.
485 doi:10.1093/imammb/dqr008
- 486 Gong, X., Lin, C., Cheng, J., Su, J., Zhao, H., Liu, T., Wen, X., Zhao, P., 2015. Generation of Multicellular Tumor Spheroids with
487 Microwell-Based Agarose Scaffolds for Drug Testing. *PLoS One* 10, e0130348. doi:10.1371/journal.pone.0130348
- 488 Goodman, T.T., Chen, J., Matveev, K., Pun, S.H., 2008. Spatio-temporal modeling of nanoparticle delivery to multicellular tumor
489 spheroids. *Biotechnol. Bioeng.* 101, 388–399. doi:10.1002/bit.21910
- 490 Helmlinger, G., Netti, P.A., Lichtenbeld, H.C., Melder, R.J., Jain, R.K., 1997. Solid stress inhibits the growth of multicellular tumor
491 spheroids. *Nat. Biotechnol.* 15, 778–83. doi:10.1038/nbt0897-778
- 492 Hengst, L., Dulic, V., Slingerland, J.M., Lees, E., Reed, S.I., 1994. A cell cycle-regulated inhibitor of cyclin-dependent kinases. *Proc.*
493 *Natl. Acad. Sci. U. S. A.* 91, 5291–5. doi:10.1073/pnas.91.12.5291
- 494 Hossain, S.S., Hossainy, S.F. a., Bazilevs, Y., Calo, V.M., Hughes, T.J.R., 2012. Mathematical modeling of coupled drug and drug-
495 encapsulated nanoparticle transport in patient-specific coronary artery walls. *Comput. Mech.* 49, 213–242.
496 doi:10.1007/s00466-011-0633-2
- 497 Jain, R.K., Martin, J.D., Stylianopoulos, T., 2014. The role of mechanical forces in tumor growth and therapy. *Annu. Rev. Biomed.*
498 *Eng.* 16, 321–46. doi:10.1146/annurev-bioeng-071813-105259
- 499 Kim, M., Gillies, R.J., Rejniak, K. a, 2013. Current Advances in Mathematical Modeling of Anti-Cancer Drug Penetration into Tumor
500 Tissues. *Front. Oncol.* 3, 278. doi:10.3389/fonc.2013.00278
- 501 Kim, T.-H., Mount, C.W., Gombotz, W.R., Pun, S.H., 2010. The delivery of doxorubicin to 3-D multicellular spheroids and tumors in a
502 murine xenograft model using tumor-penetrating triblock polymeric micelles. *Biomaterials* 31, 7386–97.
503 doi:10.1016/j.biomaterials.2010.06.004
- 504 Kim, Y., Stolarska, M. a., Othmer, H.G., 2011. The role of the microenvironment in tumor growth and invasion. *Prog. Biophys. Mol.*
505 *Biol.* 106, 353–79. doi:10.1016/j.pbiomolbio.2011.06.006
- 506 Lewis, R.W., Schrefler, B.A., 1998. The finite element method in the static and dynamic deformation and consolidation of porous
507 media. John Wiley.
- 508 Loessner, D., Flegg, J. a, Byrne, H.M., Clements, J. a, Huttmacher, D.W., 2013. Growth of confined cancer spheroids: a combined
509 experimental and mathematical modelling approach. *Integr. Biol.* 5, 597. doi:10.1039/c3ib20252f
- 510 Luk, C.K., Veinot-Drebot, L., Tjan, E., Tannock, I.F., 1990. Effect of transient hypoxia on sensitivity to doxorubicin in human and
511 murine cell lines. *J. Natl. Cancer Inst.* 82, 684–92. doi:10.1093/jnci/82.8.684
- 512 Mascheroni, P., Stigliano, C., Carfagna, M., Boso, D.P., Preziosi, L., Decuzzi, P., Schrefler, B.A., 2016. Predicting the growth of
513 glioblastoma multiforme spheroids using a multiphase porous media model. *Biomech. Model. Mechanobiol.* 15, 1215–28.
514 doi:10.1007/s10237-015-0755-0
- 515 Mikhail, A.S., Eetezadi, S., Allen, C., 2013. Multicellular tumor spheroids for evaluation of cytotoxicity and tumor growth inhibitory

516 effects of nanomedicines in vitro: a comparison of docetaxel-loaded block copolymer micelles and Taxotere®. *PLoS One* 8,
517 e62630. doi:10.1371/journal.pone.0062630

518 Montel, F., Delarue, M., Elgeti, J., Vignjevic, D., Cappello, G., Prost, J., 2012. Isotropic stress reduces cell proliferation in tumor
519 spheroids. *New J. Phys.* 14, 55008. doi:10.1088/1367-2630/14/5/055008

520 Mpekris, F., Angeli, S., Pirentis, A.P., Stylianopoulos, T., 2015. Stress-mediated progression of solid tumors: effect of mechanical
521 stress on tissue oxygenation, cancer cell proliferation, and drug delivery. *Biomech. Model. Mechanobiol.* 14, 1391–402.
522 doi:10.1007/s10237-015-0682-0

523 Mueller-Klieser, W., 2000. Tumor biology and experimental therapeutics. *Crit. Rev. Oncol. Hematol.* 36, 123–39.

524 Mueller-Klieser, W., Freyer, J.P., Sutherland, R.M., 1986. Influence of glucose and oxygen supply conditions on the oxygenation of
525 multicellular spheroids. *Br. J. Cancer* 53, 345–53.

526 Mueller-Klieser, W.F., Sutherland, R.M., 1982. Oxygen tensions in multicell spheroids of two cell lines. *Br. J. Cancer* 45, 256–64.

527 Netti, P.A., Berk, D.A., Swartz, M.A., Grodzinsky, A.J., Jain, R.K., 2000. Role of extracellular matrix assembly in interstitial transport in
528 solid tumors. *Cancer Res.* 60, 2497–503.

529 Pinder, G.F., Gray, W.G., 2008. *Essentials of Multiphase Flow and Transport in Porous Media*, 1st ed. John Wiley & Sons, Hoboken,
530 New Jersey. doi:10.1002/9780470380802

531 Planas-Silva, M.D., Weinberg, R.A., 1997. The restriction point and control of cell proliferation. *Curr. Opin. Cell Biol.* 9, 768–72.
532 doi:10.1016/S0955-0674(97)80076-2

533 Polyak, K., Kato, J.Y., Solomon, M.J., Sherr, C.J., Massague, J., Roberts, J.M., Koff, A., 1994. p27Kip1, a cyclin-Cdk inhibitor, links
534 transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev.* 8, 9–22. doi:10.1101/gad.8.1.9

535 Preziosi, L., Ambrosi, D., Verdier, C., 2010. An elasto-visco-plastic model of cell aggregates. *J. Theor. Biol.* 262, 35–47.
536 doi:10.1016/j.jtbi.2009.08.023

537 Preziosi, L., Tosin, A., 2009. Multiphase and multiscale trends in cancer modelling. *Math. Model. Nat. Phenom.* 4, 1–11.
538 doi:10.1051/mmnp/20094301

539 Sciumè, G., Gray, W.G., Ferrari, M., Decuzzi, P., Schrefler, B.A., 2013. On Computational Modeling in Tumor Growth. *Arch. Comput.*
540 *Methods Eng.* 20, 327–352. doi:10.1007/s11831-013-9090-8

541 Sciumè, G., Shelton, S., Gray, W., Miller, C., Hussain, F., Ferrari, M., Decuzzi, P., Schrefler, B., 2013. A multiphase model for three-
542 dimensional tumor growth. *New J. Phys.* 15, 15005. doi:10.1088/1367-2630/15/1/015005

543 Seebacher, N.A., Richardson, D.R., Jansson, P.J., 2015. Glucose modulation induces reactive oxygen species and increases P-
544 glycoprotein-mediated multidrug resistance to chemotherapeutics. *Br. J. Pharmacol.* 172, 2557–2572.
545 doi:10.1111/bph.13079

546 St Croix, B., Flørenes, V.A., Rak, J.W., Flanagan, M., Bhattacharya, N., Slingerland, J.M., Kerbel, R.S., 1996. Impact of the cyclin-
547 dependent kinase inhibitor p27Kip1 on resistance of tumor cells to anticancer agents. *Nat. Med.* 2, 1204–10.

548 St Croix, B., Sheehan, C., Rak, J.W., Flørenes, V. a, Slingerland, J.M., Kerbel, R.S., 1998. E-Cadherin-dependent growth suppression is
549 mediated by the cyclin-dependent kinase inhibitor p27(KIP1). *J. Cell Biol.* 142, 557–71. doi:10.1083/jcb.142.2.557

550 Stylianopoulos, T., Martin, J.D., Chauhan, V.P., Jain, S.R., Diop-Frimpong, B., Bardeesy, N., Smith, B.L., Ferrone, C.R., Hornicek, F.J.,
551 Boucher, Y., Munn, L.L., Jain, R.K., 2012. Causes, consequences, and remedies for growth-induced solid stress in murine and
552 human tumors. *Proc. Natl. Acad. Sci.* 109, 15101–15108. doi:10.1073/pnas.1213353109

553 Trédan, O., Galmarini, C.M., Patel, K., Tannock, I.F., 2007. Drug resistance and the solid tumor microenvironment. *J. Natl. Cancer*
554 *Inst.* 99, 1441–54. doi:10.1093/jnci/djm135

555 Vinci, M., Gowan, S., Boxall, F., Patterson, L., Zimmermann, M., Court, W., Lomas, C., Mendiola, M., Hardisson, D., Eccles, S. a, 2012.
556 Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation
557 and drug evaluation. *BMC Biol.* 10, 29. doi:10.1186/1741-7007-10-29

558 Wang, X., Zhen, X., Wang, J., Zhang, J., Wu, W., Jiang, X., 2013. Doxorubicin delivery to 3D multicellular spheroids and tumors based
559 on boronic acid-rich chitosan nanoparticles. *Biomaterials* 34, 4667–79. doi:10.1016/j.biomaterials.2013.03.008

560 Ward, J.P., King, J.R., 2003. Mathematical modelling of drug transport in tumour multicell spheroids and monolayer cultures. *Math.*
561 *Biosci.* 181, 177–207.

562 Weinberg, B.D., Patel, R.B., Exner, A. a., Saidel, G.M., Gao, J., 2007. Modeling doxorubicin transport to improve intratumoral drug
563 delivery to RF ablated tumors. *J. Control. Release* 124, 11–9. doi:10.1016/j.jconrel.2007.08.023

564 Xing, H., Wang, S., Hu, K., Tao, W., Li, J., Gao, Q., Yang, X., Weng, D., Lu, Y., Ma, D., 2005. Effect of the cyclin-dependent kinases
565 inhibitor p27 on resistance of ovarian cancer multicellular spheroids to anticancer chemotherapy. *J. Cancer Res. Clin. Oncol.*
566 131, 511–519. doi:10.1007/s00432-005-0677-9

567 Zahreddine, H., Borden, K.L.B., 2013. Mechanisms and insights into drug resistance in cancer. *Front. Pharmacol.* 4, 28.
568 doi:10.3389/fphar.2013.00028

