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INVESTIGATING THE STEADY STATE OF MULTICELLULAR SPHEROIDS BY REVISITING THE TWO-FLUID MODEL

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Abstract. In this paper we examine the steady state of tumour spheroids considering a structure in which the central necrotic region contains an inner liquid core surrounded by dead cells that keep some mechanical integrity. This partition is a consequence of assuming that a finite delay is required for the degradation of dead cells into liquid. The phenomenological assumption of constant local volume fraction of cells is also made. The above structure is coupled with a simple mechanical model that views the cell component as a viscous fluid and the extracellular liquid as an inviscid fluid. By imposing the continuity of the normal stress throughout the whole spheroid, we show that a steady state can exist only if the forces on cells at the outer boundary (provided e.g. by a surface tension) are intense enough, and in such a case we can compute the stationary radius. By giving reasonable values to the parameters, the model predicts that the stationary radius decreases with the external oxygen concentration, as expected from experimental observations.

1. **Introduction.** In recent years it has become more and more apparent that any realistic approach to cancer modelling has to take into account the mechanical interactions among the various components (cells, extracellular liquid, extracellular matrix, etc.) [11, 10, 2, 23, 13, 1], besides all the complex aspects related to cell metabolism [27, 3, 5]. Of course the crucial element in the formulation of a mathematical model of a tumour in the framework of mixture theory is the selection of constitutive laws for the various components and for their mutual interactions. The price to pay when introducing sophisticated constitutive equations is the number of parameters to be defined, for which it is sometimes very difficult (if not impossible) to provide more than a guess of the order of magnitude.

Avoiding the intricacy of the full description of forces is sometimes possible when dealing with multicellular spheroids, which are the simplest possible tumour structures and that can be grown in suspensions or in gels under controlled conditions. Although the presence of some extracellular matrix has been evidenced [18], in vitro spheroids are usually modelled by considering just two volume filling components: cells and extracellular liquid, frequently supposed to have the same density. When

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their shape is reasonably approximated by a sphere (cases of symmetry loss are also possible, see [15]), the introduction of suitable assumptions, like imposing the cell volume fraction and a rate for the degradation of dead cells (a limit assumption is the immediate disappearance of dead cells), results in a radical simplification, since in that case the mechanics enters the model only at the kinematical level [8, 9, 28, 29, 6]. In other words, mass balance becomes sufficient to determine the velocity fields of cells and of the extracellular liquid, without referring to any constitutive law for the mixture. This way of approaching the problem has the obvious advantage of simplicity, but it is based on radical and arbitrary assumptions.

The opposite point of view is to attempt to describe the dynamics of the mixture, which necessarily brings in the model all the difficulties which have been already recalled.

It looks very reasonable to search for a compromise between exceedingly simple and exceedingly complicated models. In a recent paper [14] we made a first attempt in this direction, considering the specific case of spheroids at a steady state. In such a condition, which can be experimentally observed, the new cells produced in the proliferating rim generate a cell flow towards the central necrotic region, accompanied by a flow of the extracellular liquid in the opposite direction (the latter flow provides the material needed to proliferation). In [14] we have emphasized the importance of modelling the distribution of the necrotic material. The model illustrated there assumed the presence of a liquid necrotic core, surrounded by a region in which dead cells still keep some integrity, so that the mechanical properties can be considered in practice identical to the ones of the viable rim (this extreme situation was obtained supposing that the conversion of dead cells from "solid" to "liquid" takes a fixed time). Cells (alive or dead) were supposed to occupy a given volume fraction, but no further assumption of kinematical type have been made. This view, which somehow parallels the approach of [22], contrasts models in which the necrotic core is filled by dead cells keeping a constant local volume fraction while dissolving into liquid with a given rate. In the latter case all components are known to have zero velocity at the centre, but for the model including a liquid necrotic core the global flux continuity condition at the solid/liquid interface is not sufficient to close the system and to determine the stationary radius. The missing equation was provided in [14] by imposing that at equilibrium the power dissipated in the motion of the two components has to be balanced by the mechanical power produced by the proliferating cells, which act as the engine forcing the two flows in opposite directions. Clearly the computation of the dissipated power requires the choice of a mechanical model for the mixture. Here comes the compromise, since we have adopted a simple model in the framework of the two-fluid approach. Two-fluid schemes are certainly not close to reality, but they have the obvious advantage of introducing a minimal number of parameters. We point out that, since the approach of [14] contemplates a constant cell volume fraction, it deviates from the two-fluids models usually found in the literature, which try to describe cell-cell interactions by means of a potential depending on the local average cell-cell distance [10, 2]. However, the ensuing simplification allows some explicit computation and a far easier interpretation of the results.

In order to focus on the mechanical condition closing the system, in [14] we confined the chemical side of the model to the diffusion-consumption of oxygen. Having calculated the power dissipated at the steady state, we made the simplifying assumption that all proliferating cells, in any point of the spheroid and under

any growth condition, produce the same amount of (mechanical) energy. In that way the total energy produced by cells in the unit time was proportional to the volume occupied by proliferating cells. To make calculation even simpler, we adopted a model with thresholds of oxygen concentration for proliferation, quiescence and death, so that we had regions separated by sharp interfaces. The power produced by the individual proliferating cell was calculated with reference to a known experimental condition and then used to predict the spheroid size corresponding to different external oxygen concentrations. The results showed to follow the expected trend, known from experimental observations [17].

Despite its promising appearance (energy balance is an undisputable physical principle), the model of [14] still relies on the arbitrary assumption that proliferating cells provide the same amount of mechanical power, independently of their local conditions. This conjecture is obviously questionable. Can we say anything more rigorous? In the present paper we keep basically the same model as [14], but we replace the conjecture about the mechanical power delivered by proliferating cell with the more natural requirement that the normal stress is everywhere continuous. In particular this amounts to imposing the normal stress continuity at the interface where the mixture composition is discontinuous, namely the boundary of the liquid central core. The new model still predicts the reduction of the stationary radius with the decrease of the external oxygen concentration and, remarkably, shows that the existence of a steady state *requires* suitable forces acting selectively on the cells at the outer boundary. Though the theory here developed does not support the approach of [14], we shall see that in the practical cases considered the discrepancies are rather small.

2. **Modelling the internal structure of a multicellular spheroid at the steady state.** For our purposes it is convenient to divide the stationary spheroid into spherically symmetric domains, separated by sharp interfaces. The introduction of such free boundaries complicates the mathematical structure of the problem, but it provides a considerable conceptual simplification. As we did in [14], we consider just oxygen as the limiting nutrient, so oversimplifying the description of metabolism, in order to concentrate on the mechanical aspects. The partition of the spheroid is obtained by introducing thresholds for the oxygen concentration $\sigma(r)$, where r is the radial coordinate varying between zero and the unknown spheroid radius R. More precisely, we have a proliferation threshold σ_P and a necrosis threshold $\sigma_N < \sigma_P$, so that all cells in the region $P = \{r : \sigma(r) > \sigma_P\}$ are proliferating (with the rate χ), while the cells in the region $Q = \{r : \sigma_N < \sigma(r) < \sigma_P\}$ are quiescent. The necrotic region $N = \{r : \sigma(r) \leq \sigma_N\}$ is also partitioned into two sub-domains NS and NL: in NS cells are supposed to keep the mechanical properties they had upon entering the necrotic region, while the inner core NL is simply liquid. The latter partition follows from the assumption that the cell membrane takes some given time τ_D to degrade and that its degradation marks the transition from "solid" to "liquid". Of course this point of view is rather extreme, and various modifications could be proposed. We note here that nuclear magnetic resonance (NMR) measurements of the self-diffusion of water in EMT-6 spheroids [25] showed that whereas in the viable rim water appears to be confined into two compartments with different diffusion coefficients (intracellular and extracellular water), the central necrotic core looks as a single compartment characterised by a single diffusion coefficient. Moreover, NMR imaging evidenced an intermediate zone between the

viable rim and the center of the necrotic region, and this intermediate zone appeared too to have two diffusion compartments, although the fraction of volume of the diffusion-restricted compartment was found lower than the corresponding fraction in the viable rim.

The above scheme of a stationary spheroid includes three interfaces:

- $r = \rho_P$, the $P - Q$ interface;
- $r = \rho_N$, the $Q - N$ interface;
- $r = \rho_D$, the $NS - NL$ interface.

The determination of ρ_P, ρ_N goes through the solution of the following *oxygen diffusion-consumption problem*: given the radius R of the spheroid, find a twice continuously differentiable function $\sigma(r)$, and ρ_P, ρ_N, such that

$$D\Delta\sigma(r) = f(\sigma(r))\nu, \qquad \text{in } P, \tag{1}$$

$$D\Delta\sigma(r) = \frac{1}{m} f(\sigma(r))\nu, \qquad \text{in } Q, \tag{2}$$

$$\sigma(R) = \sigma^* > \sigma_P, \tag{3}$$

$$\sigma(\rho_P) = \sigma_P, \tag{4}$$

$$\sigma(\rho_N) = \sigma_N, \tag{5}$$

$$\sigma'(\rho_N) = 0. \tag{6}$$

Here D is the oxygen diffusivity in the spheroid, $\Delta = \frac{1}{r^2}\frac{d}{dr}\left(r^2\frac{d}{dr}\right)$ is the Laplacian operator, $f(\sigma(r))$ is a Michaelis-Menten type consumption rate in P, reduced by the factor $1/m < 1$ in Q, ν is the constant cell volume fraction in P and Q, and σ^* is the given oxygen concentration at the exterior. This problem is not trivial, but it can be proved that (see [4])

- for any given R sufficiently large there exists one and only one solution (otherwise the solution does not exist),
- the differences $R - \rho_P$, $\rho_P - \rho_N$ tend to stabilize, beyond some value of R, to values depending on σ^*.

In order to find ρ_D it is necessary to calculate the velocity field $\mathbf{u}$ of the cells in $P \cup Q \cup NS$. We suppose that the cell volume fraction ν keeps in NS the same value as in P and Q, and we assume the same mass density for the cells and the liquid. The saturation condition implies that $1 - \nu$ is the liquid volume fraction. Denoting by $\mathbf{v}$ the liquid velocity field, we have the system

$$\nabla \cdot \mathbf{u} = \chi, \qquad \text{in } P, \tag{7}$$

$$\nabla \cdot \mathbf{u} = 0, \qquad \text{in } Q \cup NS, \tag{8}$$

$$\nabla \cdot \mathbf{v} = -\chi\frac{\nu}{1-\nu}, \qquad \text{in } P, \tag{9}$$

$$\nabla \cdot \mathbf{v} = 0, \qquad \text{in } Q \cup N, \tag{10}$$

which keeps into account the incompressibility of the mixture, i.e we have $\nabla \cdot [\nu\mathbf{u} + (1-\nu)\mathbf{v}] = 0$. Moreover both $\mathbf{u}$ and $\mathbf{v}$ are radially directed. By imposing the global flux continuity at $r = \rho_D$

$$\mathbf{v}(\rho_D^-) = \nu\mathbf{u}(\rho_D^+) + (1-\nu)\mathbf{v}(\rho_D^+),$$

since (10) together with $\mathbf{v}(0) = 0$, which holds by symmetry, imply $\mathbf{v}(\rho_D^-) = 0$, we get

$$\nu\mathbf{u}(\rho_D^+) + (1-\nu)\mathbf{v}(\rho_D^+) = 0,$$

so that for any $r \in (\rho_D, R)$ we have

$$\nu\mathbf{u} + (1-\nu)\mathbf{v} = 0, \tag{11}$$

i.e. a global no flux condition holds (therefore, at the steady state both $\mathbf{u}$ and $\mathbf{v}$ vanish at $r = R$). Note that having taken the same density for the cells and for the liquid, proliferation and degradation do not imply volume changes.

Since $\mathbf{u}$ is zero on $r = R$ at the steady state, the radial component $u(r)$ of the cell velocity can be easily computed giving:

$$u(r) = -\frac{\chi}{3r^2}(R^3 - r^3), \qquad \text{for } \rho_P < r < R, \tag{12}$$

$$u(r) = -\frac{\chi}{3r^2}(R^3 - \rho_P^3), \qquad \text{for } \rho_D < r < \rho_P. \tag{13}$$

The latter formula emphasizes the occurrence of a singularity if ρ_D is allowed to vanish. Following the motion along the velocity field (13), we can deduce the value of ρ_D imposing that

$$\tau_D = -\int_{\rho_D}^{\rho_N} \frac{dr}{u(r)},$$

so that ρ_D is given by

$$\rho_D^3 = \rho_N^3 - \chi\tau_D(R^3 - \rho_P^3), \tag{14}$$

which represents a constraint on the system, meaning that R has to be sufficiently large to allow the latter equation to have a positive solution. In other words, through (13) and (14) we recognize that a transition of cells from the "solid" to the "liquid" phase that occurs with a fixed delay from death is not compatible (at the steady state) with a necrotic core fully "solid", if we want to avoid that the cell velocity goes to infinity at $r = 0$.

We write the right hand side of (1) in the form $nQ\sigma(r)/(H + \sigma(r))$, where Q is the maximum oxygen consumption rate per cell and n is the cell concentration. In the following simulations we will take $Q = 8.3 \cdot 10^{-17}$mol/(cell· sec) [16], $n = 5 \cdot 10^8$cell/cm^3 [16], $H = 4.64 \cdot 10^{-3}$mM [12], $m = 2$ [7], $D = 1.82 \cdot 10^{-5}$cm^2/sec [24], $\sigma_P = 0.05$ mM, $\sigma_N = 0.01$ mM, $\chi = \log 2/48$ h^{-1}, $\tau_D = 48$ h.

Fig. 1 shows the radii ρ_P, ρ_N, ρ_D as functions of R, in case of $\sigma^* = 0.28$ mM [17]. The difference $R - \rho_N$ and $\rho_N - \rho_D$ stabilize for R large enough.
At this point it is clear that the internal structure of the stationary spheroid can be found once R is known. To proceed further for determining R we must address the mechanical issue.

3. **A mechanical scheme based on the two-fluid model.** As we said, we select the two-fluid model for its simplicity, keeping the additional constraint that the cell volume fraction ν is everywhere constant. According to such an approach cells are described as a Newtonian viscous fluid and the extracellular liquid as an inviscid fluid. There is a general agreement (see [10], [23]) on the following form of the Cauchy stress tensors for the two components:

$$\mathbf{T}_C = \nu\big[-p_C\mathbf{I} + 2\eta_C\mathbf{D}_C - \frac{2}{3}\eta_C\nabla\cdot\mathbf{u}\mathbf{I}\big] \tag{15}$$

$$\mathbf{T}_E = (1-\nu)\big[-p_E\mathbf{I}\big] \tag{16}$$

where $\mathbf{D}_C$ is the strain rate tensor of the cellular component, η_C is the "viscosity" of cells (bringing cell-cell interactions in the model), p_C, p_E, are the pressures in the respective components. In view of (7), (8) we have $\nabla\cdot\mathbf{u} = \chi_P(r)$, where $\chi_P(r) = \chi$ in the region P and vanishes otherwise. Although the two components

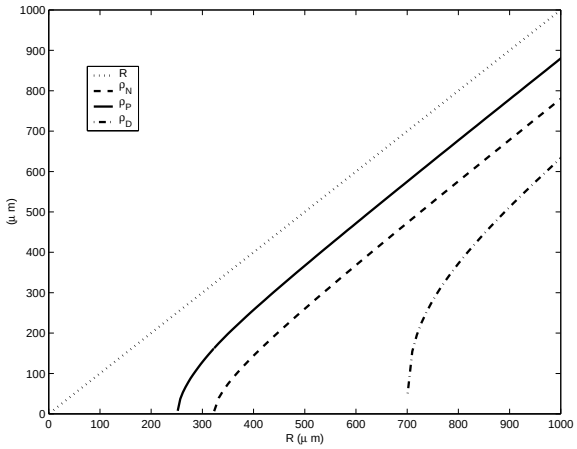

FIGURE 1. ρ_P, ρ_N, ρ_D as functions of R. Parameter values indicated in the text.

are incompressible, the mass exchange accompanying proliferation makes $\nabla \cdot \mathbf{u}$ (and $\nabla \cdot \mathbf{v}$) different from zero in the proliferation rim. This explains the structure of $\mathbf{T}_C$. It is important to distinguish between the two pressures p_C, p_E since it is possible to apply different normal stresses to each component at the outer surface. We recall that in spherical coordinates and in spherical symmetry the strain rate tensor has the form $\mathbf{D}_C = \mathrm{Diag}[u',\ u/r,\ u/r]$.

As in [14], we write the momentum balance equations in the form

$$\delta\nu\frac{d\mathbf{u}}{dt} = \nabla \cdot \mathbf{T}_C + \mathbf{m}_C, \tag{17}$$

$$\delta(1-\nu)\frac{d\mathbf{v}}{dt} = \nabla \cdot \mathbf{T}_E + \mathbf{m}_E, \tag{18}$$

where $\mathbf{m}_C$, $\mathbf{m}_E$ are the interaction forces between the components, which are assumed to be proportional to the relative velocity $\mathbf{v} - \mathbf{u}$,

$$\mathbf{m}_C = \lambda_C(\mathbf{v} - \mathbf{u}), \tag{19}$$

$$\mathbf{m}_E = \lambda_E(\mathbf{u} - \mathbf{v}), \tag{20}$$

and δ is the common density of the components.

The explicit expression of such forces can be found by imposing that

(i) the overall momentum exchange rate is zero (a customary assumption in mixture theory [26]):

$$0 = \mathbf{m}_C + \chi_P\delta\nu\mathbf{u} + \mathbf{m}_E - \chi_P\delta\nu\mathbf{v} = \frac{\lambda_E - \lambda_C}{1-\nu}\mathbf{u} + \frac{\chi_P\delta\nu}{1-\nu}\mathbf{u}. \tag{21}$$

The momentum exchange is indeed due to direct interaction, through $\mathbf{m}_C$, $\mathbf{m}_E$, and to mass exchange;

(ii) the liquid flow is governed by the Darcy's law:

$$\mathbf{v} - \mathbf{u} = -K\nabla p_E, \tag{22}$$

where $K(1-\nu)$ is the hydraulic conductivity of the spheroid.

These assumptions yield the formulae

$$\mathbf{m}_C = -\left(\frac{1}{K} + \chi_P \delta \frac{\nu}{1-\nu}\right)\mathbf{u}, \tag{23}$$

$$\mathbf{m}_E = \frac{\mathbf{u}}{K}. \tag{24}$$

In practice $\mathbf{m}_C = -\mathbf{u}/K$, since the other term is absolutely negligible (and so is the contribution of mass exchange to the momentum exchange rate).

Inertia is likewise negligible, so that the left hand side of equations (17), (18) can be replaced with zero. The equations so modified can be looked at as the differential equations determining the unknown pressures p_C, p_E in terms of R and of the data prescribed on the outer surface. After some algebra (see [14] for details) we get indeed:

$$p_C' = -\frac{u}{K\nu} + \frac{4}{3}\eta_C \chi \delta(r - \rho_P), \tag{25}$$

$$p_E' = \frac{u}{K(1-\nu)}, \tag{26}$$

where $\delta(\cdot)$ denote the Dirac function.

4. **Pressure fields and normal stress continuity.** By integrating (25),(26), where u is expressed by (12),(13), we obtain:

$$p_E(r) = p_{\text{ext}} + \frac{\chi}{3K(1-\nu)}\left(\frac{R^3}{r} + \frac{r^2}{2} - \frac{3}{2}R^2\right), \qquad \text{for } \rho_P \le r \le R, \tag{27}$$

$$p_E(r) = p_E(\rho_P) + \frac{\chi(R^3 - \rho_P^3)}{3K(1-\nu)}\left(\frac{1}{r} - \frac{1}{\rho_P}\right), \qquad \text{for } \rho_D \le r < \rho_P, \tag{28}$$

$$p_C(r) = \hat{p} + \frac{2\gamma}{R} - \frac{\chi}{3K\nu}\left(\frac{R^3}{r} + \frac{r^2}{2} - \frac{3}{2}R^2\right), \qquad \text{for } \rho_P < r < R, \tag{29}$$

$$p_C(r) = p_C(\rho_P^-) - \frac{\chi(R^3 - \rho_P^3)}{3K\nu}\left(\frac{1}{r} - \frac{1}{\rho_P}\right), \qquad \text{for } \rho_D < r < \rho_P, \tag{30}$$

where the jump

$$p_C(\rho_P^+) - p_C(\rho_P^-) = \frac{4}{3}\eta_C \chi, \tag{31}$$

is produced by the presence of the Dirac function in equation (25), in turn generated by the discontinuity of $\nabla \cdot \mathbf{u}$. Note that $p_E(r)$ is decreasing and $p_C(r)$ is increasing for $r \in (\rho_D, R]$. In (27), p_{ext} is the external pressure exerted on the extracellular liquid. The term $2\gamma/R$ in (29) is due to the so-called "tumour surface tension" (denoted by γ), and the sum $\hat{p} + 2\gamma/R$ is the total pressure acting on cells at the outer surface. $\hat{p}$ is in general greater than p_{ext} and includes for example the reaction of the surrounding medium which has been deformed by the growth of the spheroid. This is the typical situation in gels. It has been shown [19] that an increase of the culture gel density results in a reduction of the equilibrium size of spheroids. The rigorous derivation of $\hat{p}$ should include the analysis of the stress in the surrounding material, and the condition of continuity of the normal stress at $r = R$. Thus $\hat{p}$ would include also a contribution originated by the flow itself, which appears nevertheless to be modest if the cellular viscosity is in the range we have considered. Since we do not want to deal with the mechanics of the exterior

domain, here we simply set $\hat{p} = p_{\text{ext}}$, though this question is not trivial and would deserve a larger discussion.

Remark 1. The physical origin of a surface tension effect in a spheroid is not quite the same as that of the corresponding phenomenon observed in a liquid drop. In the latter case surface tension is generated by intermolecular forces in the presence of curvature. Cells instead interact through macromolecular bridges which can be formed or destroyed to accommodate internal evolution. Attraction forces are produced upon stretching of these intercellular bonds and the resulting normal stress can be considered proportional to curvature only if the latter is not too large. In any case, the underlying mechanism seems hardly compatible with the "cellular fluid" model, but we still consider it a necessary compromise. □

Let us turn to our original goal which was to impose the continuity of the normal component of the stress. The critical interface on which we have to write down this condition is $r = \rho_D$. In the inner liquid core the stress is uniform and isotropic, reducing to the pressure $p_E(\rho_D)$, which is continuous across the interface. The solid fraction contribution to the normal stress is

$$-\nu p_C + 2\nu\eta_C\,\vec{\mathbf{e}}\cdot\mathbf{D}_C\vec{\mathbf{e}}, \tag{32}$$

where $\vec{\mathbf{e}} = \vec{\mathbf{r}}/r$ is the unit normal vector. Since $\vec{\mathbf{e}}\cdot\mathbf{D}_C\vec{\mathbf{e}} = u'$, the continuity of the normal stress takes the form

$$\nu p_C(\rho_D^+) - 2\nu\eta_C u'(\rho_D^+) + (1-\nu)p_E(\rho_D) = p_E(\rho_D), \tag{33}$$

that is

$$p_C(\rho_D^+) = p_E(\rho_D) + 2\eta_C u'(\rho_D^+). \tag{34}$$

Taking into account (27)-(31), we obtain the final condition

$$\begin{aligned}\frac{2\gamma}{R} =& \frac{1}{\nu(1-\nu)}\frac{\chi R^2}{3K}\left\{\frac{R}{\rho_D}\left[1-\left(\frac{\rho_P}{R}\right)^3\right]-\frac{3}{2}\left[1-\left(\frac{\rho_P}{R}\right)^2\right]\right\}\\ &+\frac{4}{3}\eta_C\chi\left\{1+\left(\frac{R}{\rho_D}\right)^3\left[1-\left(\frac{\rho_P}{R}\right)^3\right]\right\},\end{aligned} \tag{35}$$

which is the desired equation for the determination of R. In this equation, ρ_P and ρ_D are function of R after the solution of problem (1)-(6) and thank to (14). We recall that to the left hand side we may add any additional normal stress exerted selectively on cells at the outer surface.

Remark 2. When K is sufficiently small the viscosity term in (35) can be neglected and the two parameters γ, K enter (35) only through their product. □

Remark 3. When the normal stress is continuous at $r = \rho_D$, from (34) we see that $p_C(r)$ is greater than $p_E(r)$ for any r in $(\rho_D, R]$. □

It is convenient to rewrite (35) in the form

$$\begin{aligned}2\gamma =& \frac{1}{\nu(1-\nu)}\frac{\chi R^3}{3K}\left\{\frac{R}{\rho_D}\left[1-\left(\frac{\rho_P}{R}\right)^3\right]-\frac{3}{2}\left[1-\left(\frac{\rho_P}{R}\right)^2\right]\right\}\\ &+\frac{4}{3}\eta_C\chi R\left\{1+\left(\frac{R}{\rho_D}\right)^3\left[1-\left(\frac{\rho_P}{R}\right)^3\right]\right\},\end{aligned} \tag{36}$$

and to remark that

(i) the right hand side tends to infinity when R approaches the value for which ρ_D vanishes, as well as when R tends to infinity;
(ii) the right hand side has one (and only one) positive minimum, which we will denote by $2\gamma^*$;
(iii) for $\gamma < \gamma^*$ equation (36) has no solutions. For $\gamma > \gamma^*$ it has two solutions, but the physical one is the smaller (for a spheroid that has grown to the steady state from a small size).

A conclusion which seems quite acceptable is therefore that *no steady state solution exists if the external action on the spheroid is not intense enough.* The external action can be provided by the so-called surface tension or by external forces acting on the cells. This prediction agrees with the results of [22]. When equation (36) has no solution we must infer that the spheroid grows indefinitely. Of course such statement makes sense only in the framework of the present model and as long as the chosen boundary conditions can be applied. How to adapt the model to describe the evolution of a nonstationary spheroid will be the objective of a forthcoming paper.

5. **Numerical results.** In [14] we considered, as reference case, a spheroid having the stationary radius of 1 mm when cultured with $\sigma^* = 0.28$ mM. In that case we chose $\nu = 0.6$, $K = 10^{-7}\text{cm}^3 \cdot \text{sec/g}$ and $\eta_C = 10^4$ Poise, and we estimated the power w_P supplied by proliferating cells per unit cell volume, obtaining $w_P = 9.05 \cdot 10^{-7}\text{g/(cm} \cdot \text{sec}^3)$. For the hydraulic conductivity measured in tumours, values two or three orders of magnitude less have been reported [21]. However, we may observe that the cell number density and the ECM content of *in vivo* tumours are likely to be higher than in *in vitro* spheroids. The chosen value of the cell viscosity was suggested by measurements reported in [20]. The above values proved to make the cell-cell friction and the liquid-cell friction contributes to power dissipation of comparable magnitude.

Keeping the above values for ν, K, η_C, we look for γ such that a value of R close to 1 mm solves (36) when $\sigma^* = 0.28$ mM. This procedure is illustrated in Fig. 2: with $\gamma = 0.0107$ dyne/cm the equation (36) is solved by $R = 984$ μm.

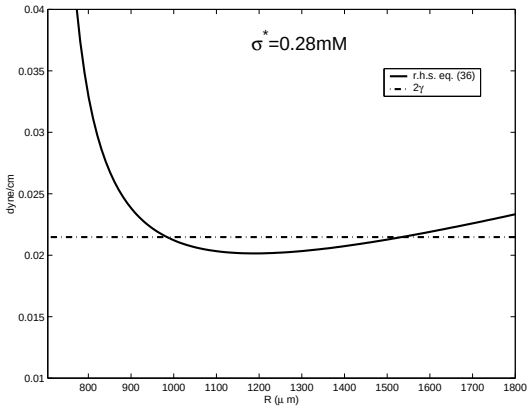

FIGURE 2. Profile of the r.h.s. of (36) as function of R, for $\sigma^* = 0.28$ mM. The predicted stationary radius is $R = 984$ μm .

With $\gamma = 0.0107$ dyne/cm, different values of σ^* yield the results in Fig. 3. The values in Table 1 reproduce the expected trend of the radius of the stationary

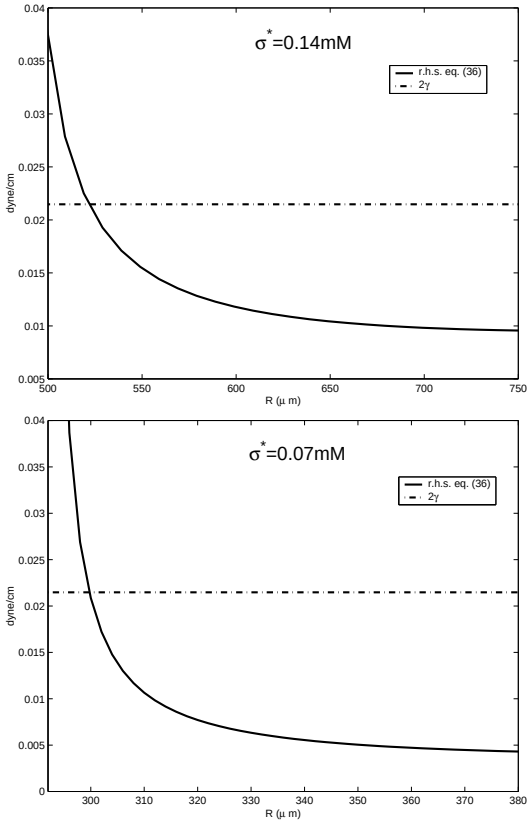

FIGURE 3. Profiles of the r.h.s. of (36) as function of R, for different values of σ^*. The predicted stationary radii are $R = 522$ μm (top) and $R = 300$ μm (bottom).

spheroid. Though the results are not identical with those of [14], they appear equally realistic.

We may explore different situations, taking for instance a case in which the liquid-cell friction dominates ($K = 10^{-8}$ cm$^3 \cdot$ sec/g). In such a case the value $\gamma = 0.0527$ dyne/cm predicts $R = 971$ μm at the steady state for $\sigma^* = 0.28$ mM. With this value of γ, different values of σ^* yield the results in Table 1.

It is interesting to make a comparison with the results of [14] based on the conjecture that any proliferating cell supplies the same power. Clearly, the two approaches do not look compatible, since the dissipated power (as calculated in [14] on the basis of the known velocity fields) is not dependent on γ and so will be the stationary radius determined according to [14]. Nevertheless, in the range considered, the differences in prediction may be not large. For $K = 10^{-7}$ cm$^3 \cdot$ sec/g, $\sigma^* = 0.28$ mM and $R = 984$ μm, we have computed the dissipated power and the power per unit cell volume, obtaining $w_P = 9.67 \cdot 10^{-7}$ g/(cm $\cdot$ sec^3). Using this value, by equalling the dissipated power and the power supplied by proliferating cells we predicted at $\sigma^* = 0.14$ mM and $\sigma^* = 0.07$ mM stationary radii that differ

K	γ	σ^*	Radius at the steady state
$10^{-7} cm^3 \cdot sec/g$	$0.0107\, dyne/cm$	$0.28\, mM$	$R = 984\, \mu m$
		$0.14\, mM$	$R = 522\, \mu m$
		$0.07\, mM$	$R = 300\, \mu m$
$10^{-8} cm^3 \cdot sec/g$	$0.0527\, dyne/cm$	$0.28\, mM$	$R = 971\, \mu m$
		$0.14\, mM$	$R = 493\, \mu m$
		$0.07\, mM$	$R = 293.5\, \mu m$

TABLE 1. Radius of the spheroid at the steady state, for different values of σ^*, K, and γ.

less than 15% from those predicted by the present approach. The same was done for $K = 10^{-8}$ cm$^3 \cdot$ sec/g, $\sigma^* = 0.28$ mM and $R = 971$ μm, obtaining $w_P = 4.30 \cdot 10^{-6}$ g/(cm $\cdot$ sec^3) and stationary radii even closer to those of Table 1.

6. **Modelling cells as a Bingham fluid.** Since intercellular links break beyond some stress, it has been suggested that the Newtonian fluid modelling cells could be replaced by a Bingham fluid (see [1]).

Let us see how the results of the previous section modify if we adopt this option, keeping the same symbol for viscosity and introducing the yield stress τ_0. The cell stress tensor is now

$$\mathbf{T_C} = -\nu \left(p_C + \frac{2}{3}\eta_C \nabla \cdot \mathbf{u} \right) \mathbf{I} + \nu \boldsymbol{\tau} \tag{37}$$

where

$$\boldsymbol{\tau} = \left(2\eta_C + \frac{\tau_0}{\sqrt{II_{D_C}}} \right) \mathbf{D}_C \tag{38}$$

if $\sqrt{II_\tau} > \tau_0$, and $\mathbf{D_C} = 0$ otherwise. For a tensor $\mathbf{A}$, we define the frame invariant quantity II_A as

$$II_A = \frac{1}{2} Tr \mathbf{A}^2. \tag{39}$$

Thus, we find that

$$\sqrt{II_\tau} = 2\eta_C \sqrt{II_{D_C}} + \tau_0, \qquad \text{if } II_{D_C} > 0. \tag{40}$$

Let us compute II_{D_C},

$$II_{D_C} = \frac{u'^2}{2} + \frac{u^2}{r^2}, \tag{41}$$

which on $r = \rho_D$ gives

$$II_{D_C} = 3\frac{u^2}{\rho_D^2}, \tag{42}$$

implying

$$\boldsymbol{\tau} = \left(2\eta_C + \frac{\tau_0}{\sqrt{3}\frac{|u|}{\rho_D}} \right) \mathbf{D}_C, \qquad \text{for } r = \rho_D. \tag{43}$$

The radial projection of $\boldsymbol{\tau}$ at $r = \rho_D$ is then $4\eta_C \frac{|u|}{\rho_D} + 2\frac{\tau_0}{\sqrt{3}}$. Therefore the normal stress continuity condition on $r = \rho_D$ is now

$$\nu p_C - 2\nu\eta_C u' - 2\nu \frac{\tau_0}{\sqrt{3}} + (1-\nu) p_E = p_E. \tag{44}$$

Imposing the same condition at $r = R$, we conclude that (35) modifies to:

$$\frac{2\gamma}{R} = \frac{1}{\nu(1-\nu)}\frac{\chi R^2}{3K}\left\{\frac{R}{\rho_D}\left[1-\left(\frac{\rho_P}{R}\right)^3\right]-\frac{3}{2}\left[1-\left(\frac{\rho_P}{R}\right)^2\right]\right\}$$
$$+\frac{4}{3}\eta_C\chi\left\{\left(\frac{R}{\rho_D}\right)^3\left[1-\left(\frac{\rho_P}{R}\right)^3\right]\right\}+(\frac{2}{\sqrt{3}}-\sqrt{2})\tau_0. \tag{45}$$

Again the solution we are looking for is the minimal root. It is immediate to realize that, if the remaining parameters are not changed, the presence of τ_0 has two effects: it lowers the minimal surface tension for the existence of the steady state, and if (45) has a solution it is smaller than the solution of (35). For τ_0 to be influential, if e.g. γ/R is of the order of 10^{-1} dyne/cm^2, it must be at least of the order of 10^{-2}dyne/cm^2, which happens to be the same order of magnitude of $p_E(0) - p_{ext}$ (see [14]).

We may remark that both the surface tension and the yield stress have their physical origin in the intercellular adhesion bonds. Therefore it is possible that the surface tension γ has a monotone dependence on the yield stress τ_0. In that case there can be a partial amplification of the effect of the yield stress on the determination of R.

7. **Concluding remarks.** In this paper we examined the steady state of tumour spheroids considering a structure in which the central necrotic region contains an inner liquid core surrounded by dead cells that keep some mechanical integrity. This partition is a consequence of assuming that a finite delay is required for the degradation of dead cells into liquid. The phenomenological assumption of constant local volume fraction of the cell component is also made. The above structure is combined with a simple mechanical model that views the cell component as a viscous fluid and the extracellular liquid as an inviscid fluid. By imposing the continuity of the normal stress throughout the whole spheroid, we show that a steady state can exist only if the forces on cells at the outer boundary (provided e.g. by a surface tension) are intense enough. In such a case we can compute the stationary radius. The key mechanical parameters are the cell viscosity, the hydraulic conductivity, and the surface tension coefficient. By giving reasonable values to these parameters, the model predicts that the stationary radius decreases with the external oxygen concentration as expected from experimental observations.

The same mechanical scheme was studied in a previous paper [14], in which the power supplied by each proliferating cells was introduced as a parameter characterising all proliferating cells, and the stationary radius was computed by balancing the dissipated power with the supplied power. The two approaches do not give the same results (although the results can be numerically similar) because the assumption that the mechanical power supplied by the individual cells is equal for all the cells, irrespective of their local conditions, is clearly an oversimplification. We guess that the results could be made congruent by linking the power supplied by a cell to the component of the local cell-cell interaction which is expressed, in the two-fluid model, by the difference between the cell pressure and the extracellular liquid pressure.

As a general remark, we note that the two-fluid approach can be substantially generalised. Since cells may adapt their mutual interactions to the local dynamical conditions, one could let the "cell viscosity" depend e.g. on p_C (of course this would

have some influence only when the system is not liquid-dominated). Making the two-fluid picture more flexible could allow to formulate conjectures on the mechanical properties of the spheroid, without losing the continuity of the normal stress. For instance, it would be possible to investigate the possibility that the steady state radius corresponds to a minimum of the dissipated power (which is certainly not true for the simple scheme here adopted). As we said in the introduction, the price to pay for having more flexibility is the introduction of more parameters, which should always be physically meaningful. The extension proposed in Sect.6 goes in that direction and is indeed promising,

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REFERENCES

[1] D. Ambrosi and L. Preziosi, *Cell adhesion mechanisms and stress relaxation in the mechanics of tumours*, Biomech. Model. MechanoBiol., **8** (2009), 397–413.

[2] S. Astanin and L. Preziosi, *Multiphase models of tumour growth*, in "Selected Topics in Cancer Modelling" (eds. N. Bellomo, M. Chaplain and E. De Angelis), Birkhauser, (2008), 223–250.

[3] S. Astanin and L. Preziosi, *Mathematical modelling of the Warburg effect in tumour cords*, J. Theor. Biol., **258** (2009), 578–590.

[4] A. Bertuzzi, A. Fasano and A. Gandolfi, *A free boundary problem with unilateral constraints describing the evolution of a tumour cord under the influence of cell killing agents*, SIAM J. Math. Analysis, **36** (2004), 882–915.

[5] A. Bertuzzi, A. Fasano, A. Gandolfi and C. Sinisgalli, *Necrotic core in EMT6/Ro tumor spheroids: Is it caused by an ATP deficit?*, J. Theor. Biol., **262** (2010), 142–150.

[6] A. Bertuzzi, C. Bruni, A. Fasano, A. Gandolfi, F. Papa and C. Sinisgalli, *Response of tumor spheroids to radiation: Modeling and parameter identification*, Bull. Math. Biol., **72** (2010), 1069–1091.

[7] A. Bredel-Geissler, U. Karbach, S. Walenta, L. Vollrath and W. Mueller-Klieser, *Proliferation-associated oxygen consumption and morphology of tumour cells in monolayer and spheroid culture*, J. Cell. Physiol., **153** (1992), 44–52.

[8] H. M. Byrne and M. A. J. Chaplain, *Growth of nonnecrotic tumors in the presence and absence of inhibitors*, Math. Biosci., **130** (1995), 151–181.

[9] H. M. Byrne and M. A. J. Chaplain, *Growth of necrotic tumors in the presence and absence of inhibitors*, Math. Biosci., **135** (1996), 187–216.

[10] H. Byrne and L. Preziosi, *Modeling solid tumor growth using the theory of mixtures*, Math. Med. Biol., **20** (2003), 341–366.

[11] H. M. Byrne, J. R. King, D. L. S. McElwain and L. Preziosi, *A two-phase model of solid tumour growth*, Appl. Math. Lett., **16** (2003), 567–573.

[12] J. J. Casciari, S. V. Sotirchos and R. M. Sutherland, *Variation in tumor cell growth rates and metabolism with oxygen concentration, glucose concentration, and extracellular pH*, J. Cell. Physiol., **151** (1992), 386–394.

[13] V. Cristini, X. Li, J. S. Lowengrub and S. M. Wise, *Nonlinear simulations of solid tumor growth using a mixture model: invasion and branching*, J. Math. Biol., **58** (2009), 723–763.

[14] A. Fasano, A. Gandolfi and M. Gabrielli, *The energy balance in stationary multicellular spheroids*, Far East J. Math. Sci., **39** (2010), 105–128.

[15] A. Friedman and F. Reitich, *Symmetry-breaking bifurcations of analytic solutions to free boundary problems: An application to a model of tumor growth*, Trans. Amer. Math. Soc., **353** (2000), 1587–1634.

[16] J. P. Freyer and R. M. Sutherland, *A reduction in the in situ rates of oxygen and glucose consumption of cells in EMT6/Ro spheroids during growth*, J. Cell. Physiol., **124** (1985), 516–524.

[17] J. P. Freyer and R. M. Sutherland, *Regulation of growth saturation and development of necrosis in EMT6/Ro multicellular spheroids by the glucose and oxygen supply*, Cancer Res., **46** (1986), 3504–3512.
[18] G. Hamilton, *Multicellular spheroids as an in vitro tumor model*, Cancer Lett., **131** (1998), 29–34.
[19] G. Helmlingen, P. A. Netti, H. C. Lichtembeld, R. J. Melder and R. K. Jain, *Solid stress inhibits the growth of multicellular tumor spheroids*, Nature Biotech., **15** (1997), 778–783.
[20] A. Iordan, A. Duperray and C. Verdier, *A fractal approach to the rheology of concentrated cell suspensions*, Phis. Rev. E, **77** (2008), 011911.
[21] P. A. Netti and R. K. Jain, *Interstitial transport in solid tumours*, in "Cancer Modelling and Simulation" (ed. L. Preziosi), Chapman&Hall/CRC, (2003), 51–74.
[22] K. A. Landman and C. P. Please, *Tumour dynamics and necrosis: surface tension and stability*, IMA J. Math. Appl. Med. Biol., **18** (2001), 131–158.
[23] G. Lemon, J. R. King, H. M. Byrne, O. E. Jensen and K. M. Shakesheff, *Mathematical modelling of engineered tissue growth using a multiphase porous flow mixture theory*, J. Math. Biol., **52** (2008), 571–594.
[24] W. Mueller-Klieser, *Method for the determination of oxygen consumption rates and diffusion coefficients in multicellular spheroids*, Biophysical Journal, **46** (1984), 343–348.
[25] M. Neeman, K. A. Jarrett, L. O. Sillerud and J. P. Freyer, *Self-diffusion of water in multicellular spheroids measured by magnetic resonance microimaging*, Cancer Res., **51** (1991), 4072–4079.
[26] K. R. Rajagopal and L. Tao, "Mechanics of Mixtures," World Scientific, Singapore, 1995.
[27] K. Smallbone, R. A. Gatenby, R. J. Gillies, P. K. Maini and D. J. Gavaghan, *Metabolic changes during carcinogenesis: Potential impact on invasiveness*, J. Theor. Biol., **244** (2007), 703–713.
[28] J. P. Ward and J. R. King, *Mathematical modelling of avascular tumor growth I*, IMA J. Math. Appl. Med. Biol., **14** (1997), 36–69.
[29] J. P. Ward and J. R. King, *Mathematical modelling of avascular tumor growth II. Modelling growth saturation*, IMA J. Math. Appl. Med. Biol., **16** (1999), 171–211.

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