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THE STEADY STATE OF MULTICELLULAR TUMOUR SPHEROIDS: A MODELLING CHALLENGE

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Abstract

Cells from many tumour cell lines can be grown in vitro to form spheroidal aggregates, called multicellular tumour spheroids, currently considered valuable experimental models of avascular tumours. During the spheroid growth the fraction of proliferating cells decreases, and when cells in the inner region become deprived of nutrients, and/or metabolic waste accumulates, cell death occurs. Thus, in a late stage of growth, the spheroids consist of an outer viable rim surrounding a central necrotic region, whereas the spheroid growth, which is initially exponential, tends to saturate. In the present paper, we first give a brief survey of the modelling options proposed in the literature for describing the necrotic core and for explaining the steady state of tumour spheroids. Then, we review and reconsider our recent work on this topic, based on the two-fluid mechanical scheme, energy balance, and stress analysis.

1. Introduction

Cells from different tumour cell lines can be grown *in vitro* to form spheroidal masses, called multicellular tumour spheroids, currently considered valuable experimental models of avascular tumours [56, 48, 35, 49]. Multicellular tumour spheroids have been extensively investigated in that they provide a useful model to assess the effects of oxygenation and nutrition on growth, as well as the effects of treatments with drugs and radiation.

During the spheroid growth the fraction of proliferating cells decreases, and when cells in the inner region become deprived of oxygen, glucose and other nutrients, and/or metabolic waste accumulates, cell death occurs. Thus, in a late stage of growth, the spheroids consist of an outer viable rim (whose thickness takes values from about $100\,\mu$m to $250\,\mu$m) surrounding a central necrotic region. The spheroid growth is initially exponential and then it tends to saturate. Examples of reaching the stationary state (with final diameter of 1-3 mm) have been reported [30, 32].

Many mathematical models have been proposed to describe the spheroid evolution, from simple growth models such as Gompertzian models [44], to models that take into account the internal spheroid structure using either continuum or discrete approaches (see [6] for an extensive review). In almost all these models, cell proliferation and death are assumed to depend on the concentration of a single critical chemical (generally oxygen), diffusing from the external medium into the spheroid mass. According to this view, the boundary between the viable rim and the necrotic core is often defined as the level set of the oxygen concentration corresponding to a given threshold. However, the formation mechanism of the central necrotic region in multicellular spheroids is a much debated and a not yet well understood process. The diffusion of both glucose and oxygen has been included in the spheroid models proposed in [21, 38, 53]. More recently, the cell energy metabolism, i.e. the intracellular ATP production involving glucose, oxygen and lactate, has been incorporated in models of spheroids [59, 10, 11], as well as in various models of tumour growth [8, 7, 55, 33]. In [10, 11], the formation of the sheroid necrotic region was described by assuming that cell death occurs when the ATP production rate falls below a critical value. The possible role of acidity in determining the onset of the central necrosis in tumours was investigated in [13].

With only a few exceptions [46, 3, 4], the final attainment of a steady state during the sheroid growth has been associated, in the modelling literature, with a loss of volume from the necrotic core that balances the new cellular volume created in the viable rim by cell proliferation. The experimental evidence of this mechanism, however, is indirect, and relies on the observation of active cell proliferation even when the growth rate of the spheroid is very small or vanishes [27]. Such a general tenet has been declined within different, sometimes contrasting, descriptions of the central necrotic region. From a biological point of view the way the necrotic core is modelled may look to be a minor question. Nevertheless, the structure attributed to the necrotic zone has a crucial influence on the general mechanical behaviour of the entire spheroid and hence on its evolution.

In the present paper, we first want to offer a brief survey (Section 2) of the modelling options proposed in the literature for describing the necrotic core and for explaining the steady state of tumour spheroids. Then, we review and reconsider our recent work on this topic (Section 3 - Section 5). Some concluding remarks are given in Section 6.

2. A Brief History of the Necrotic Core Modelling

In the influential paper by Greenspan [34], the necrotic core, composed of 'dead cells and cellular material in various stages of disintegration' is viewed as a 'jelly-like' material 'capable of supporting the pressure exerted on it by the outer viable layers'. This "solid" debris, while keeping a constant density, continually dissolves into 'simpler permeable compounds' capable of moving easily through the outer region of the spheroid. Because of this motion, a volume loss occurs from the region of necrosis. Although it does not play a direct role in the model, a surface tension is postulated to maintain the compactness of the aggregate. The degradation of the necrotic material occurs according to a first order kinetics, with uniform degradation rate constant through the whole core. In Greenspan's model, however, the volume loss is not the only mechanism allowing the attainment of a steady state: another important role is played by a mitosis inhibitor which is supposed to be produced at a constant rate in the necrotic core, or as a

waste from living cells (for comments about this conjecture, see [5]). Cell death occurs when the oxygen concentration decreases to some critical threshold (a feature incorporated in many subsequent models) and mitosis stops when the inhibitor concentration raises above the inhibition threshold. According to this picture, the model contains two free boundaries. A substantial gap of the model is the absence of any mechanical explanation of how the postulated material loss from the necrotic core can take place. In other words, a study of the flow of the various components based on the general principles of mechanics is missing. This kind of analysis came much later in cancer modelling.

The Greespan's viewpoint was largely adopted in the following years (see Deakin [26], MacElwain and Ponzo [45], Maggelakis and Adam [42], Adam and Maggelakis [5], Byrne and Chaplain [17], Cui and Friedman [23], Bertuzzi *et al.* [12]), and in the next Section we will illustrate a simple model based on it. Also the model studied by Cui and Friedman in [24], describes the central zone of a spherical tumour (although without a sharp interface), as essentially full of dead cells subjected to degradation according to a uniform rate constant.

A different mechanism for the attainment of a stationary state during the spheroid growth was proposed by McElwain and Morris [46]. These authors, following Burton [16], assumed the necrotic material immune from degradation (at least in the time horizon of interest) and supposed that the relevant volume loss happens in the inner viable rim via cell apoptosis and phagocytosis of the resulting apoptotic bodies by the viable neighboring cells. This mechanism then accounts for some experimental observations of stationary spheroids without central necrosis [57]. Volume loss was totally absent in the models by Adam [3] and Adam and Maggelakis [4], where instead a diffusing endogenous mitotic inhibitor, possibly produced inside the necrotic region [4], eventually blocks the proliferation of all the cells.

Some different views of the necrotic region derived from the explicit modelling of the multi-phase nature of the cell aggregates. Ward and King [60] distinguished in the spheroid the viable cells (that can occupy a varying local volume fraction) and a diffusible 'cellular material' originated by the immediate degradation of cells upon death (a death rate is introduced depending on the concentration of a critical chemical). This material may be reused to sustain the cell proliferation, so that growth saturation can be achieved. The necrotic core is then a zone deprived of cells but occupied by the cellular material, and the volume loss from the necrotic core is given by the diffusing flow of such a material towards the outer region. This view was reconsidered in [58], where, however, the lack of reutilization of the material coming from cell disintegration prevents the saturation of growth.

Following some ideas of the model in [52], Landman and Please [40] described the spheroid as a liquid-cells mixture whose mechanics is borrowed from a model for suspensions [41]. The force balance equation is explicitly included together with the mass balance, and not only the liquid, but also the cell component has isotropic stress tensor. Thus, stresses are expressed by two pressures: the liquid pressure and the intercellular pressure. The net proliferation and death rate is expressed as a function of oxygen concentration, switching sign across a critical threshold. Cells immediately degrade into liquid after death (then all cells are living cells), whereas maintaining a constant local volume fraction (and a compact arrangement) until the cellular pressure is greater than the liquid pressure. Complete mass exchange between liquid and cellular phase occurs during cell proliferation and at cell death. An interesting feature introduced in [40] is claiming that when the cellular pressure tends to drop below the liquid pressure, cells detach from the compact arrangement and "float" in the liquid. Thus the necrotic core is essentially described as a liquid with a small fraction of viable cells committed to death. This fraction vanishes at the steady state, when the necrotic core is purely liquid. However, the existence of the steady state is related to the presence of a suitable surface tension: if the surface tension is insufficient, the spheroid eventually will grow linearly.

A two-phase model based on a more complex mechanics was proposed by Byrne and Preziosi [18] (see also [1, 15, 19]). In this "two-fluid" model, cells are represented by a viscous fluid whose pressure contains an extra-term depending on the cell volume fraction and describing the cell-to-cell interaction, whereas the extracellular liquid is represented by an inviscid fluid. Again, in this model cells degrade istantaneously into liquid after death, and complete mass exchange between liquid and cellular phase occurs at cell proliferation and cell death, which are under the control of a critical nutrient. At the steady state, the local volume fraction of (living) cells continuously decreases toward the center of the spheroid, at which it does not vanishes, so that the necrotic core is mimicked by a region in which the

Table 1: Main features of the description of the necrotic core (N) in some literature models. Volume loss: [a] postulated; [b] by diffusion; [c] supported by a fluid dynamical theory.

Model	**Dead cell degradation**	**Necrotic core structure**	**Reaching steady state**	**Mechanical aspects**
[34]	first order kinetics, mitotic inhibitor production	solid	volume loss from N [a] + mitotic inhibition	mono-phase model, kinematic approach
[46]	absent	solid	phagocytosis of apoptotic cells	mono-phase model, kinematic approach
[4]	absent, mitotic inhibitor production	solid	proliferation blocked by mitotic inhibitors diffusing from N	mono-phase model, kinematic approach
[60]	immediate	reservoir of diffusible "cellular material"	volume loss from N [b]	two-phase model (one phase diffusible)
[40]	immediate	liquid at the steady state	volume loss from N [c]	two-fluid model, momentum balance, surface tension (needed for equilibrium)
[18]	immediate	reduced density of living cells	volume loss from N [c]	two-fluid model, momentum balance, surface tension may be included
[12]	after a Gamma-distributed time	solid	volume loss from N [a]	multi-phase model, kinematic approach
[29]	after a fixed time	includes a liquid core within a solid shell	volume loss from N [c]	two-fluid model, kinematic approach + stress analysis, surface tension (needed for equilibrium)

density of living cells is reduced. A similar view is also present in the model by Ambrosi and Preziosi [2], in which the cell component is represented by a visco-elasto-plastic fluid, and in the model proposed by Cristini *et al.* [25], focussed on the derivation of the interaction potential.

In two recent papers [28, 29], we have proposed that at the steady state the necrotic core (N) may be partitioned in two zones: a "solid" domain NS where dead cells are supposed to keep the mechanical properties they had before death and the same volume fraction, and an inner core NL simply liquid. This partition follows from the assumption that there is a finite maximal time taken by the cell membrane to degrade and that this degradation marks the transition from "solid" to "liquid". In fact, the existence of such maximal time contradicts that dead cells can be present with non-zero volume fraction in all the necrotic core at any time, since in that case all the trajectories of the material points in N would approach the center in an infinite time because their velocity would have to vanish at the center by symmetry. Note that when a first order kinetics is assumed for cell degradation, since the degradation time is implicitly supposed to be exponentially distributed, such a time has not a finite upper bound. For simplicity, in [28, 29] a fixed degradation time was assumed.

Some support to the NS/NL partition comes from nuclear magnetic resonance (NMR) measurements of the self-diffusion of water in EMT-6 spheroids [50]. These measurements have shown that whereas in the viable rim water appears confined into two compartments with different diffusion coefficients (intracellular and extracellular water), the central part of the necrotic core looks like 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, that still appears 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 presence of an inner liquid core precludes, however, the possibility of studying the spheroid on a pure kinematic basis, and requires the introduction of a mechanical scheme. In the above cited papers, the two-fluid model was revisited to investigate the spheroid steady-state .

The main features of the necrotic core description in some literature models are summarized in Table 1.

3. The Internal Structure of a Multicellular Spheroid

Let us assume the spheroid be a mixture of two component, cells and extracellular liquid, whose velocities are denoted respectively by $\mathbf{u}$ and $\mathbf{v}$. The local volume fractions of living cells, dead cells and extracellular liquid, are denoted by ν_C, ν_N and ν_E respectively. Assuming no voids, we have $\nu_C + \nu_N + \nu_E = 1$. We will consider oxygen as the limiting nutrient, so oversimplifying the description of metabolism in order to concentrate on the mechanical aspects. In spherical symmetry, we denote by $\sigma(r,t)$ the oxygen concentration, r being the radial distance from the spheroid center and t the time. Let $R(t)$ be the outer radius of the spheroid. To gain some conceptual simplification, it is convenient to divide the spheroid into spherically symmetric domains, separated by sharp interfaces. The partition of the spheroid is obtained by introducing thresholds for the oxygen concentration. More precisely, we introduce a proliferation threshold σ_P, and a necrosis threshold $\sigma_N < \sigma_P$ assuming that all cells die when the oxygen concentration reaches σ_N. So, all cells in the region $P = \{r : \sigma(r,t) > \sigma_P\}$ are proliferating, while the cells in the region $Q = \{r : \sigma_N < \sigma(r,t) < \sigma_P\}$ are quiescent. The necrotic region is given by $N = \{r : \sigma(r,t) = \sigma_N\}$. We will assume that in P and in Q it is $\nu_C = \nu = constant$. For simplicity, we take that all cells in P consume oxygen at the same rate, and proliferate with a common constant proliferation rate χ. The above scheme of a spheroid includes two interfaces:

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

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

$$D_{O_2}\Delta\sigma(r) \quad = \quad f(\sigma(r))\nu, \qquad \text{in } P, \tag{1}$$

$$
\begin{aligned}
D_{O_2}\Delta\sigma(r) &= \frac{1}{m}f(\sigma(r))\nu, \qquad \text{in } Q, && (2)\\
\sigma(R) &= \sigma^*, && (3)\\
\sigma(\rho_P) &= \sigma_P, && (4)\\
\sigma(\rho_N) &= \sigma_N, && (5)\\
\sigma_r(\rho_N) &= 0. && (6)
\end{aligned}
$$

In the above equations, D_{O_2} 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 the consumption rate per unit cell volume in P, reduced by the factor $1/m < 1$ in Q, and σ^* is the given oxygen concentration at the external boundary ($\sigma^* > \sigma_P$). This problem is not trivial, but it can be proved (with the techniques of [9]) that:

- for any given R sufficiently large there exists one and only one solution $\rho_P = \hat{\rho}_P(R)$, $\rho_N = \hat{\rho}_N(R)$ (otherwise at least one of the interface does not exist),
- the differences $R - \rho_P$, $\rho_P - \rho_N$ tend to stabilize, as R increases, to values depending on σ^* and obtainable by solving the much simpler system in plane geometry.

3.1. The "Solid" Necrotic Core Model

We give here a short description of a simple model based on a two-phase approach, assuming, as in Greenspan [34], that the necrotic core is "solid". The necrotic core will be then filled by dead cells whose local volume fraction ν_N is constant while they are dissolving into liquid with a rate constant μ_N. Supposing that all the components of the mixture have equal mass density and that $\nu_N = \nu$, the mass balance yields the following equations for $\mathbf{u}$ and $\mathbf{v}$:

$$
\begin{aligned}
\nabla\cdot\mathbf{u} &= \chi, && \text{in } P, && (7)\\
\nabla\cdot\mathbf{u} &= 0, && \text{in } Q, && (8)\\
\nabla\cdot\mathbf{u} &= -\mu_N, && \text{in } N, && (9)\\
\nabla\cdot\mathbf{v} &= -\chi\frac{\nu}{1-\nu}, && \text{in } P, && (10)\\
\nabla\cdot\mathbf{v} &= 0, && \text{in } Q, && (11)\\
\nabla\cdot\mathbf{v} &= \mu_N\frac{\nu}{1-\nu}, && \text{in } N. && (12)
\end{aligned}
$$

By multiplying Eqs. (7)-(9) by ν and (10)-(12) by $1-\nu$, and summing up, we obtain

$$\nabla\cdot(\nu\mathbf{u} + (1-\nu)\mathbf{v}) = 0, \tag{13}$$

both in $P\cup Q$ and in N.

In spherical symmetry the velocities are expressed by the scalars $u(r,t)$ and $v(r.t)$, and symmetry imposes

$$u(0,t) = 0, \qquad v(0,t) = 0. \tag{14}$$

Therefore, taking into account the continuity of the velocities at $r = \rho_N(t)$ and at $r = \rho_P(t)$, from (7)-(9) and (14), the following expression for u can be obtained:

$$
u(r,t) = \begin{cases} -\frac{\mu_N}{3}r, & \text{in N}\\ -\frac{\mu_N}{3}\frac{\rho_N^3(t)}{r^2}, & \text{in Q}\\ \frac{\chi}{3}\left(r - \frac{\rho_P^3(t)}{r^2}\right) - \frac{\mu_N}{3}\frac{\rho_N^3(t)}{r^2}, & \text{in P}\end{cases} \tag{15}
$$

From (13) and (14) it follows that

$$v(r,t) = -\frac{\nu}{1-\nu}u(r,t).$$

The evolution of the outer radius is determined by the equation

$$\dot{R}(t) = u(R(t), t),$$

and a steady-state exists for the R value such that

$$\chi\big(R^3 - \hat{\rho}_P^3(R)\big) = \mu_N \hat{\rho}_N^3(R).$$

Note that at the interface $r = \rho_N$ the velocity u is negative and v is positive: so, there is continual *loss of liquid* from the necrotic core induced by the constraint $\nu_N = \nu = constant$. It can be easily verified that this volumetric loss $4\pi\rho_N^2 v(\rho_N, t)(1-\nu)$ is equal to $\frac{4}{3}\pi\rho_N^3 \nu\mu_N$. At the steady-state, it is $u(R) = v(R) = 0$, so that all the liquid mass necessary for cell proliferation comes from the necrotic core.

Assuming a first order kinetics for the degradation of dead cells corresponds to supposing that degradation occurs randomly *à la* Poisson, i.e. that the time interval from cell death to cell dissolution is exponentially distributed with mean value equal to $1/\mu_N$. In [12], a Gamma distribution for the degradation time was considered, and the distributed delay of cell dissolution was modelled by the passage of dead cells through a chain of n equal stages with Poisson exit, the last one marking the actual transition to the liquid waste. According to that model, Eq. (9) is changed into the following set of equations

$$\begin{aligned}
&\frac{\partial \nu_{N_1}}{\partial t} + \nabla\cdot(\mathbf{u}\nu_{N_1}) = -\mu'_N \nu_{N_1}\,,\\
&\frac{\partial \nu_{N_2}}{\partial t} + \nabla\cdot(\mathbf{u}\nu_{N_2}) = \mu'_N \nu_{N_1} - \mu'_N \nu_{N_2}\,,\\
&\qquad\qquad\vdots\\
&\frac{\partial \nu_{N_n}}{\partial t} + \nabla\cdot(\mathbf{u}\nu_{N_n}) = \mu'_N \nu_{N_{n-1}} - \mu'_N \nu_{N_n}\,,
\end{aligned}$$

where $\nu_{N_i}(r,t)$ is the local volume fraction of dead cells in the i-th subcompartment, $i = 1, \ldots, n$, and μ'_N is the exit rate constant from each subcompartment. For the volume fractions of cells in different stages of death, the constraint $\sum \nu_{N_i} = \nu = constant$ is assumed, so that the velocity u can still be determined by $u(0,t) = 0$.

3.2. The NS/NL Partition of the Necrotic Core

In [28, 29], as previously mentioned, we have assumed that the necrotic core at the steady state is partitioned in two zones: a "solid" domain NS where cells are supposed to keep the mechanical properties they had before death and the same volume fraction ν, and an inner liquid core NL. As we said in Section 2, the actual presence of the latter structure appears to have some experimental support. As a matter of fact, by "liquid" we mean a mixture that may contain solid fragments and macromolecules. The important feature from the mechanical point of view is that the stress, in static condition, is isotropic. In the biological literature we may find evidences of more complex states, like e.g. coagulative necrosis [43], that would require, however, much more complicated constitutive equations.

Dead cells are supposed to degrade into liquid after a fixed time, θ_D, upon death. Such an assumption makes a new interface appear, $r = \rho_D$, dividing NS from NL.

In order to find ρ_D at the stationary state, it is necessary to calculate the velocity field $\mathbf{u}$ of the cells in $P \cup Q \cup NS$. From the mass balance, we have the system

$$\nabla\cdot\mathbf{u} = \chi, \quad \text{in } P, \tag{16}$$

$$\nabla\cdot\mathbf{u} = 0, \quad \text{in } Q \cup NS, \tag{17}$$

$$\nabla\cdot\mathbf{v} = -\chi\frac{\nu}{1-\nu}, \quad \text{in } P, \tag{18}$$

$$\nabla\cdot\mathbf{v} = 0, \quad \text{in } Q \cup N, \tag{19}$$

which keeps into account the incompressibility of the mixture, i.e $\nabla\cdot[\nu\mathbf{u} + (1-\nu)\mathbf{v}] = 0$. Note that $\mathbf{u}(\rho_D, t)$ is unknown, and this fact makes it *impossible* to determine the evolution of the spheroid and the

stationary radius by means of a purely kinematic approach. This was instead possible in the case of a "solid" necrotic core because in such a case we could impose $u(0,t)=0$.

By imposing the global flux continuity at $r=\rho_D$, namely

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

since $\mathbf{v}(0)=0$, which holds by symmetry, and Eq. (19) implies $\mathbf{v}(\rho_D^-)=0$, we get

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

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

$$\nu\mathbf{u}+(1-\nu)\mathbf{v}=0, \tag{20}$$

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

$$u(r) = -\frac{\chi}{3r^2}(R^3-r^3), \qquad \text{for } \rho_P<r<R, \tag{21}$$

$$u(r) = -\frac{\chi}{3r^2}(R^3-\rho_P^3), \qquad \text{for } \rho_D<r<\rho_P. \tag{22}$$

The latter formula emphasizes the occurrence of a singularity if ρ_D is allowed to vanish. Following the motion along the velocity field (22), we can deduce the value of ρ_D imposing that

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

so that ρ_D is given by

$$\rho_D^3=\rho_N^3-\chi\theta_D(R^3-\rho_P^3). \tag{23}$$

Equation (23) represents a constraint on the system, meaning that R has to be sufficiently large to **allow (23) to have** a positive solution. Through (22) and (23) we recognize indeed that a transition from the "solid" to the "liquid" phase that occurs with a fixed delay from death is not compatible (at the steady state) with $\rho_D=0$, i.e. with a necrotic core fully "solid".

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 description of the spheroid.

4. A Mechanical Scheme Based on the Two-Fluid Model

Two-fluid models adopt the point of view that a spheroid is a two-component mixture consisting of an inviscid fluid (the extracellular fluid) and another fluid (representing cells) for which an appropriate rheological model has to be chosen. Sometimes (as in [28, 29]) a simple Newtonian scheme is assumed in which the effect of cell-cell interactions is somehow translated into a viscosity. In other models cells are treated an an inviscid fluid too [40], or according to some nonlinear constitutive law. In this Section we present the implications of identifying cells with a Newtonian fluid. The limitations which are intrinsic to this approach will be discussed in the next Section.

In the Newtonian framework, the Cauchy stress tensors for the two components are written in the form:

$$\mathbf{T}_C = \nu\big[-p_C\mathbf{I}+2\eta_C\mathbf{D}_C-\frac{2}{3}\eta_C\nabla\cdot\mathbf{u}\mathbf{I}\big] \tag{24}$$

$$\mathbf{T}_E = (1-\nu)\big[-p_E\mathbf{I}\big] \tag{25}$$

where $\mathbf{D}_C=\frac{1}{2}[\nabla\mathbf{u}+(\nabla\mathbf{u})^T]$ is the cell strain rate tensor, and η_C is the cell viscosity. In (24) the so called Stokes' assumption has been used. The pressures p_C, p_E have to stay distinct. The reason for that will

become apparent when we consider for instance the conditions at the boundary $r = R$. Each component is incompressible, but the two velocity fields $\mathbf{u}$, $\mathbf{v}$ are not divergence free in the proliferation region, as we have seen in the previous section. This leads to the definition of the discontinuous function $\hat{\chi}(r)$, equal to χ in the region P and vanishing elsewhere. It is useful to remind that in spherical coordinates and for a radial flow the tensor $\mathbf{D}_C$ has the diagonal structure Diag$(u',\ u/r,\ u/r)$.

Neglecting body forces, and denoting by $\frac{d}{dt}$ the material derivative, we write down the momentum balance equations for the two components (supposing they have the same mass density δ):

$$\delta\nu\frac{d\mathbf{u}}{dt} = \nabla\cdot\mathbf{T}_C + \mathbf{m}_C, \tag{26}$$

$$\delta(1-\nu)\frac{d\mathbf{v}}{dt} = \nabla\cdot\mathbf{T}_E + \mathbf{m}_E, \tag{27}$$

in which we define the interaction forces $\mathbf{m}_C$, $\mathbf{m}_E$ to be

$$\mathbf{m}_C = \lambda_C(\mathbf{v}-\mathbf{u}), \tag{28}$$

$$\mathbf{m}_E = \lambda_E(\mathbf{u}-\mathbf{v}). \tag{29}$$

The coefficients λ_C, λ_E can be found by imposing two conditions:

(i) the global balance of momentum exchange rate

$$0 = \mathbf{m}_C + \hat{\chi}\delta\nu\mathbf{u} + \mathbf{m}_E - \hat{\chi}\delta\nu\mathbf{v} = \frac{\lambda_E-\lambda_C}{1-\nu}\mathbf{u} + \frac{\hat{\chi}\delta\nu}{1-\nu}\mathbf{u}; \tag{30}$$

(ii) the Darcy's law for the flow of the extracellular liquid relative to cells

$$\mathbf{v}-\mathbf{u} = -K\nabla p_E, \tag{31}$$

where $K(1-\nu)$ plays the role of hydraulic conductivity.

The final result deduced from (28)-(31) is that the interaction forces have the expressions:

$$\mathbf{m}_C = -\left(\frac{1}{K} + \hat{\chi}\delta\frac{\nu}{1-\nu}\right)\mathbf{u}, \tag{32}$$

$$\mathbf{m}_E = \frac{\mathbf{u}}{K}. \tag{33}$$

In practice equation (32) reduces to $\mathbf{m}_C = -\mathbf{u}/K$ with very good approximation. Coming back to equations (26), (27), we note that the inertia terms can be neglected. It is not difficult to show that those equations provide the governing differential system for the two pressures $p_C(r)$, $p_E(r)$, namely

$$p_C' = -\frac{u}{K\nu} + \frac{4}{3}\eta_C\chi\delta(r-\rho_P), \tag{34}$$

$$p_E' = \frac{u}{K(1-\nu)}, \tag{35}$$

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

5. Looking for Steady States

5.1. General Considerations

As we said at the end of Sect. 3, if we are given the spheroid radius R, we can find all other unknowns. Thus we need just one more equation to find R. The most natural way of proceeding is to impose that the normal component of the total stress is continuous across the critical interface, i.e. $r = \rho_D$. This is the technique we used in [29].

However, before we come to that, we want to discuss the possibility that the missing equation could be derived from considerations based on power dissipation. Looking at the problem from the point of view of energy is advantageous because it highlights the relative contribution of cell-cell friction and of liquid-cell friction, ultimately related with the coefficients η_C and K, respectively.

Here, we have also the opportunity to point out some internal contradictions of the two-fluid model that we summarize in the following remarks.

Remark 5.1. *The sheer fact that we use Darcy's law to describe the motion of the extracellular fluid relative to cells implies that a fluid-cell friction does exist, though we have supposed that the fluid is inviscid (which would make it flow among cells with no resistance). But certainly its viscosity is many orders of magnitude smaller than η_C, thus the above compromise is reasonable.*

Remark 5.2. *As we have seen, in many models (including the model in [29]) the action of "surface tension" is necessary to reach equilibrium. However, we must not identify the concept of surface tension in a spheroid with the one arising in a liquid drop. Indeed cells mutually interact through macromolecular bridges which can provide some limited tensile stress and evolve according to the dynamical state of the spheroid. At the outer surface of a growing spheroid such stresses can produce an effect similar to surface tension, but only if the number of cells in the spheroid is large enough. Thus it would be wrong to use the classical Laplace formula in the early stage of the spheroid growth, introducing in the model abnormally high pressures which simply are not there. The correct way of using surface tension in spheroids should be instead to let it come into play in a gradual way as the size of the spheroid grows.*

Remark 5.3. *The action of intercellular links cannot be fully taken into account in the framework of the two-fluid model if the "cell fluid" is Newtonian. Thus, in view of the previous remark, including surface tension is in fact an internal contradiction. For this reason may be of interest to study model extensions in which the cell component is represented by a Bingham fluid (such an extension was preliminarly considered in [29]).*

Any model in which mechanics is excessively simplified cannot provide an accurate description, since the adopted governing laws are in fact trying to provide a simple representation of phenomena whose nature can be more complex. At the same time, going deeper in investigating the mechanical structure clashes inevitably with the practical impossibility of getting experimental information on the many parameters involved. The unusual freedom we are offered in choosing the mechanical framework for our model leads us to the question: what could be a scientifically honest and physically reasonable criterion to make a choice? In our opinion the accessibility of data must be a major concern. The naïve two-fluid approach has the advantage of requiring just a small number of parameters, namely η_C and K (besides surface tension). But even in that case it would be too optimistic to say that a reliable measure of those quantities is available. On the contrary, there is a serious uncertainty about their order of magnitude. This unfair situation, however, is not definitely against the two-fluid scheme, since one can try to infer acceptable values for the mechanical parameters, thanks to the fact that they are so few. by comparing the model output with experimental data. The model validation consists then in fitting more experimental data using the parameters so identified. The number of uncertain parameters grows significantly with the complexity of the model. Therefore, if on one side we must be conscious of the enormous limitations of a mechanically naïve approach, on the other we must recognize that simplicity is the basic component of a practical strategy.

5.2. The Energy Based Approach

Coming back to our initial goal of obtaining one more equation from energy considerations, we may think of different options. First of all, we must look at the spheroid in its equilibrium configuration as an "engine", in which mechanical power is produced by proliferating cells and then dissipated by the internal motion so generated. At the stationary state, indeed, cells move inwards until they reach the interface $r = \rho_D$ whereas extracellular liquid move in the opposite way, the liquid in NL staying at rest (see Sect. 3.2). A very tempting criterion is to say that the radius at equilibrium corresponds to the minimal energy

produced and dissipated. In [28], instead, we started from the general principle that the equilibrium size must guarantee the balance of the power dissipated and the one produced by proliferating cells. Note that power balance for each species can be derived from the momentum balance equations (26), (27). Indeed we can write the equations

$$\delta\nu\frac{d}{dt}\left(\frac{1}{2}\mathbf{u}\cdot\mathbf{u}\right) = \nabla\cdot(\mathbf{T}_C\cdot\mathbf{u}) - \mathbf{T}_C:\mathbf{D}_C + \mathbf{m}_C\cdot\mathbf{u}, \tag{36}$$

$$\delta(1-\nu)\frac{d}{dt}\left(\frac{1}{2}\mathbf{v}\cdot\mathbf{v}\right) = \nabla\cdot(\mathbf{T}_E\cdot\mathbf{v}) - \mathbf{T}_E:\mathbf{D}_E + \mathbf{m}_E\cdot\mathbf{v}, \tag{37}$$

which take the explicit form

$$\begin{aligned}\delta\nu\frac{d}{dt}\left(\frac{1}{2}\mathbf{u}\cdot\mathbf{u}\right) &= -\nu\mathbf{u}\cdot\nabla p_C - \frac{2}{3}\nu\eta_C\nabla\chi_P\cdot\mathbf{u}\\ &\quad +2\nu\eta_C\nabla\cdot(\mathbf{D}_C\cdot\mathbf{u}) - 2\nu\eta_C\mathbf{D}_C:\mathbf{D}_C\\ &\quad -\left(\frac{1}{K}+\hat{\chi}\delta\frac{\nu}{1-\nu}\right)\mathbf{u}\cdot\mathbf{u},\end{aligned} \tag{38}$$

$$\begin{aligned}\delta(1-\nu)\frac{d}{dt}\left(\frac{1}{2}\mathbf{v}\cdot\mathbf{v}\right) &= -(1-\nu)\mathbf{v}\cdot\nabla p_E + \frac{\mathbf{u}}{K}\cdot\mathbf{v}\\ &= -(1-\nu)\mathbf{v}\cdot\nabla p_E - \frac{\mathbf{u}\cdot\mathbf{u}}{K}\frac{\nu}{1-\nu},\end{aligned} \tag{39}$$

where again the left hand sides can be neglected. Note the presence of the term $-\frac{2}{3}\nu\eta_C\nabla\hat{\chi}\cdot\mathbf{u}$, producing a Dirac distribution centered at the interface $r=\rho_P$. Such a singularity is a consequence of the extreme schematization of the transition $P\to Q$ linked to a threshold of oxygen concentration. In a model with a gradual transition the jump of $\hat{\chi}$ would be replaced by some steep variation, corresponding to a peak in the derivative, but with no substantial change in the qualitative behaviour. A very similar remark can be made for the Laplacian of $\mathbf{u}$.

Since Eqs. (38),(39) derive from the momentum balance, in view of our purpose we do not have a new piece of information. In [28] the equations above have been used to recognize the dissipation terms, namely $w_C(r)=2\nu\eta_C\mathbf{D}_C:\mathbf{D}_C$, representing the power dissipated per unit volume because of cell-cell friction, and $w_E(r)=\mathbf{u}\cdot\mathbf{u}/[K(1-\nu)]$, due to liquid-cell friction (the other terms are either negligible, or represent power production or transmission). Dissipation due to the conversion of liquid into cells in the proliferating zone can be checked to be absolutely negligible compared to w_C and to w_E. Thus it is possible to calculate the power globally dissipated, W_{diss}, by summing the two contributions integrated over the spheroid.

The explicit expression of the cell-cell friction dissipation term is

$$w_C(r)=\begin{cases}2\nu\eta_C\left[\left(\chi-2\frac{u}{r}\right)^2+2\frac{u^2}{r^2}\right]=2\nu\eta_C\left[\chi^2-4\frac{u}{r}\chi+6\frac{u^2}{r^2}\right], & \text{in } P,\\ 12\nu\eta_C\frac{u^2}{r^2}, & \text{in } Q\cup NS.\end{cases} \tag{40}$$

Integrating over the spheroid, the global cell-cell friction dissipation power is obtained as

$$W_C=\frac{16}{9}\pi\nu\eta_C\chi^2R^3\left\{y^3-\frac{1}{2}\Big(1+\frac{1}{y^3}\Big)+\Big(1-\frac{1}{y^3}\Big)^2\left[\Big(\frac{\rho_P}{\rho_D}\Big)^3-1\right]y^3\right\}, \tag{41}$$

where $y=R/\rho_P>1$. The global contribution of liquid-cell friction is

$$\begin{aligned}W_E &= \frac{4\pi\chi^2}{9K(1-\nu)}R^5\left[y-1-\Big(1-\frac{1}{y^2}\Big)+\frac{1}{5}\Big(1-\frac{1}{y^5}\Big)\right.\\ &+ \left.\Big(1-\frac{1}{y^3}\Big)^2\Big(\frac{\rho_P}{\rho_D}-1\Big)y\right].\end{aligned} \tag{42}$$

The dissipated power $W_{diss}=W_C+W_E$ results therefore a function of R.

The idea proposed in [28] was to define the *average power production* of a proliferating cells, w_{cell}, as an independent cell *parameter*, thus computing independently the global power production, W_{prod}, as a quantity simply proportional to the volume of the proliferating region (in turn a function of R). The new equation, from which the stationary radius can be determined, was then obtained by equating $W_{diss}(R)$ to $W_{prod}(R)$, i.e. to w_{cell} multiplied by the number of proliferating cells.

In [28] we considered, as reference, a spheroid having at the steady-state $R = 1\,\mathrm{mm}$ when the outer oxygen concentration is $\sigma^* = 0.28\,\mathrm{mM}$, The selected parameter values were the following: $\nu = 0.6$, $\chi = \log 2/48\,\mathrm{h}^{-1}$, $\theta_D = 48\,\mathrm{h}$, $D_{O_2} = 1.82 \cdot 10^{-5}\,\mathrm{cm}^2/\mathrm{sec}$ [47], $\sigma_P = 0.05\,\mathrm{mM}$, $\sigma_N = 0.01\,\mathrm{mM}$, $m = 2$ [14]. The right hand side of (1) was written as $nQ\sigma(r)/(H + \sigma(r))$, where Q is the maximum oxygen consumption rate per cell and n is the cell concentration, and we assumed $Q = 8.3 \cdot 10^{-17}\,\mathrm{mol}/(\mathrm{cell\,sec})$ [31], $n = 5 \cdot 10^8\,\mathrm{cell}/\mathrm{cm}^3$ [31], $H = 4.64 \cdot 10^{-3}\,\mathrm{mM}$ [20]. In models in which the volume loss of dead cells occurred after a chain of successive stages, the mean time for this process was estimeted greater than $\sim 80\,\mathrm{h}$ by fitting growth curves of treated sheroids [12] or xenografts [54]. We chose for θ_D a shorter value, taking into account that the above estimates reflect the full process of volume loss and account also for the dynamics of the effect of treatment [54].

Concerning the choice of the parameters η_C and K, we managed to obtain values for the two quantities W_C, W_E of the same order. This was achieved by assuming $\eta_C = 10^4\,\mathrm{Poise}$ according to [37] (compared to $10^{-2}\,\mathrm{Poise}$ for water at room temperature), and $K = 10^{-7}\,\mathrm{cm}^3\,\mathrm{sec}/\mathrm{g}$ (i.e. a permeability of $10^{-9}\,\mathrm{cm}^2$, typical of a moderately permeable material). Tumours in vivo have a much lower permeability (two orders of magnitude less), as healthy tissues do [51], but that is due to a considerable compactness provided by a substantial extracellular matrix. In spheroids extracellular matrix is a much lighter structure [35], and we have even neglected its volume fraction. This justifies the assumption of a relatively large value for K. The value taken for viscosity may also look quite large (in the viscosity range of a paste). In the Newtonian scheme, however, viscosity mimics not just pure friction in the relative motion of cells, but also the influence of the forming and breaking of intercellular links (which suggest that a Bingham scheme would be more appropriate). Here we are in a domain of large uncertainty and the choices made in [28], especially for the K value, are certainly questionable. Their main motivation was to keep both kinds of dissipation in the game, waiting for the acquisition of more precise information.

Remark 5.4. *The dimensionless ratio of the factors multiplying the brackets in the expressions of W_C, W_E is $4\nu(1-\nu)\eta_C K/R^2$. A typical value for ν in spheroids is 0.6, thus when $R = 1$ mm we obtain a value close to 0.1. If the product $\eta_C K$ is reduced (which can easily be the case), then dissipation is dominated by W_E.*

The value of w_{cell} might be estimated from the knowledge of the steady state radius at a given oxygen concentration (provided the values of the other parameters are known). In [28], for instance, for a spheroid having radius $R = 1\,\mathrm{mm}$ at $\sigma^* = 0.28\,\mathrm{mM}$, we calculated $W_{diss} = 6.2 \cdot 10^{-10}\,\mathrm{erg/sec}$. On the basis of the following formula derived by equating dissipated power and produced power,

$$w_{\mathrm{cell}} = W_{\mathrm{diss}} \left(\frac{\nu V_P}{v_{\mathrm{cell}}} \right)^{-1}, \tag{43}$$

where v_{cell} is the cell volume and $V_P = \frac{4\pi}{3}\left(R^3 - \rho_P^3\right)$ is the volume of the proliferating rim, we estimated for the quantity $w_P = w_{\mathrm{cell}}/v_{\mathrm{cell}}$, namely the power supplied by proliferating cells per unit cell volume, the value $w_P = 9.05 \cdot 10^{-7}\,\mathrm{g}/(\mathrm{cm\,sec}^3)$. If we now apply our conjecture that this value represents a characteristic of the proliferating cells of that cell line, and so is irrespective of the spheroid size, then we can use equation (43) (with the estimated w_{cell}, and $W_{diss}(R) = W_C(R) + W_E(R)$) as the equation determining the spheroid radius R for values of the outer oxygen concentration σ^* different from 0.28 mM. As a sensitivity test, in our simulation we checked that increasing the estimated w_P value by 20% makes R decrease by 10%.

In Fig. 1 we plot W_{diss} and W_{prod} as functions of R, for different values of w_P. Table 2 shows the values of R for different values of σ^*, deduced by means of the criterion illustrated above and assuming $w_P = 9 \cdot 10^{-7}\,\mathrm{g}/(\mathrm{cm\,sec}^3)$), compared to the experimental values in [32].

As Table 2 shows, despite the many approximations, this method is able to reproduce the experimentally observed trend in a reasonable way.

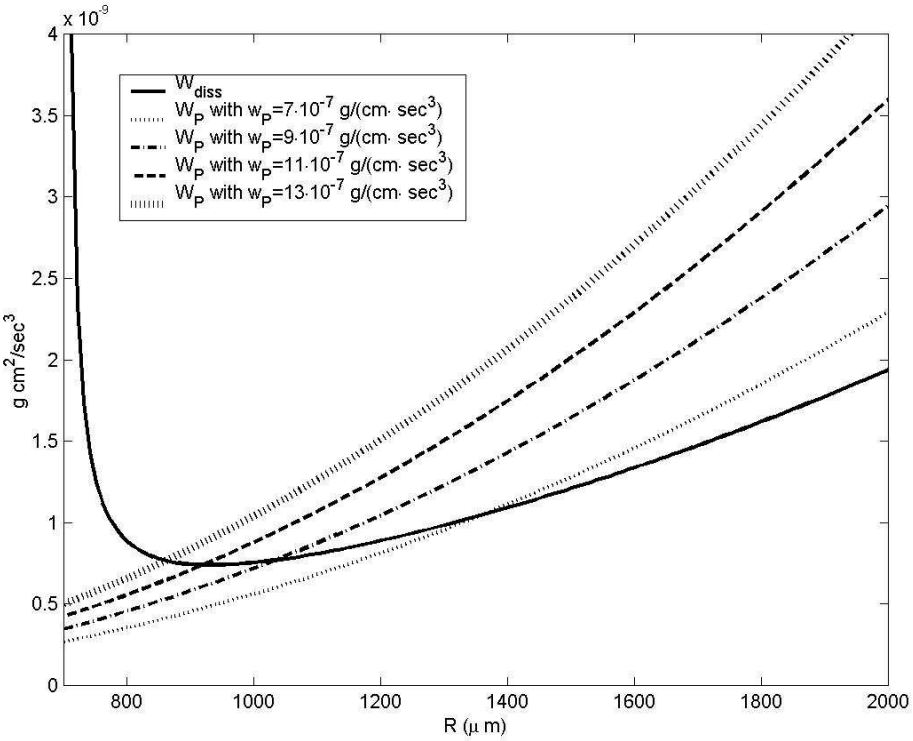

Figure 1: W_{diss} and W_{prod} for different values of w_P.

Table 2: Radius of the stationary spheroid predicted by assuming $w_P = 9 \cdot 10^{-7}\,\mathrm{g/(cm\,sec^3)}$, for different values of σ^*. The reported experimental values are the saturation values obtained by a Gompertzian fitting of the measured growth curves [32]. The glucose concentration in the medium was 5.5 mM (0.8 mM in the case of $\sigma^* = 0.07\,\mathrm{mM}$).

σ^* (mM)	Predicted radius (μm)	Experimental values (μm)
0.28	1033	1380
0.14	598	526
0.07	329	265

An interesting aspect of the analysis of mechanical power dissipation comes out by comparing w_{cell} with the power production of a tumour cell calculated on the basis of glucose consumption rate. A typical value of the glucose consumption rate in a tumour cell is $21.5 \cdot 10^{-17}$ mol/(cell sec) [31]. Since the consumption of 1 g of glucose corresponds in average to the production of 4 Kcal, we conclude that the average power production per cell is about $6.5 \cdot 10^{-3}$ erg/(cell sec). From the mechanical power dissipation we have obtained a much lower value ($w_{\rm cell} = 3.6 \cdot 10^{-15}$ erg/(cell sec), for a cell of radius 10 μm), suggesting that mechanical energy is just a very small fraction of the energy required to sustain the cell life.

5.3. Imposing Normal Stress Continuity

As we have seen, the analysis of mechanical power dissipation has some biological relevance, but it relies on the simplifying assumption that we can regard the quantity w_P as a parameter characterizing the cells of a given cell line, irrespectively of their location inside the spheroid, of the spheroid size, and of the growth conditions. Such an assumption, actually, has no biophysical justification. A different approach, having a rigorous basis in the selected mechanical framework, consists in imposing the *continuity of normal stress* throughout the spheroid. As we shall see, this approach, that has been pursued in [29], will eventually lead to rejecting (in a strict sense) the conjecture adopted in the previous section, although in some cases it may result in an acceptable approximation. In any case, the approach based on the requirement of normal stress continuity has the considerable advantage of being fully consistent with the selected mechanical model (no matter how reliable the latter is claimed to be).

In Sect. 4 we derived the differential equations (34),(35) for the pressure profile in each component. Now we perform the integration (using the expressions (21),(22) for the cell velocity field), obtaining

$$p_E(r) = p_{\rm ext} + \frac{\chi}{3K(1-\nu)}\left(\frac{R^3}{r} + \frac{r^2}{2} - \frac{3}{2}R^2\right), \quad \text{for } \rho_P \le r \le R, \tag{44}$$

$$p_E(r) = p_E(\rho_P) + \frac{\chi(R^3-\rho_P^3)}{3K(1-\nu)}\left(\frac{1}{r} - \frac{1}{\rho_P}\right), \quad \text{for } \rho_D \le r < \rho_P, \tag{45}$$

$$p_C(r) = \hat{p} + \frac{2\gamma}{R} - \frac{\chi}{3K\nu}\left(\frac{R^3}{r} + \frac{r^2}{2} - \frac{3}{2}R^2\right), \quad \text{for } \rho_P < r < R, \tag{46}$$

$$p_C(r) = p_C(\rho_P^-) - \frac{\chi(R^3-\rho_P^3)}{3K\nu}\left(\frac{1}{r} - \frac{1}{\rho_P}\right), \quad \text{for } \rho_D < r < \rho_P. \tag{47}$$

The jump

$$p_C(\rho_P^+) - p_C(\rho_P^-) = \frac{4}{3}\eta_C\chi, \tag{48}$$

is generated by the Dirac distribution in (34), that we have already commented. In (44) p_{ext} is the value taken by p_E at $r = R$, supposed to coincide with the pressure of the water component in the outer medium (which can be just water or a gel, containing in any case a preponderant water fraction). In (46) γ denotes the surface tension, and a new quantity appears, namely $\hat{p} = p_C(R) - \frac{2\gamma}{R}$, that needs some explanation. Its value has to be found by imposing the balance of normal stress when passing from the spheroid to the external medium. This operation may not be trivial.

If the spheroid is grown in water, then the external normal stress reduces to the pressure p_{ext}. If the outer medium is a gel, then on the cells there will be an extra action due to the deformation of the polymer network making the skeleton of the gel. Spheroid which are subjected to such an extra compression have been reported to exhibit a reduced growth [36]. This question would deserve a deeper investigation, since it can be related to inhibition of proliferation (which would make the proliferation χ depend on pressure [22]), but can also have an independent mechanical origin, as we shall see. It has to be emphasized that the extra compression, coming from the solid component of gel, acts only on the solid component of the spheroid (namely the cells, despite their schematization as a fluid). Similarly, surface tension acts exclusively on the cells.

Thus, while we just have pressure continuity for the liquid component, the boundary condition for p_C can be stated by imposing the following jump condition to the total stress $\mathbf{T}$, relative to radial direction

(see e.g. [39]),

$$\big[\mathbf{Te}\cdot\mathbf{e}\big]_{r=R} = -\nu\frac{2\gamma}{R}, \tag{49}$$

where $\mathbf{e}$ is the radial unit vector. Equation (49) is equivalent to

$$-\nu\big(p_C(R)+\frac{2}{3}\eta_C\chi\big)+2\nu\eta_C u'(R)-(1-\nu)p_{ext} = -\nu p_{gel}-\nu\frac{2\gamma}{R}-p_{ext}, \tag{50}$$

where we have denoted by p_{gel} the pressure selectively exerted on cells by the gel polymeric network. Such a quantity should be represented by a monotone function of R, stabilizing to an asymptotic finite value, since the network deformation can only have a somehow localized influence. Here p_{ext} is atmospheric pressure. We recall that $u'(R)=\chi$. Hence (50) implies

$$\hat{p} = p_{gel}+p_{ext}+\frac{4}{3}\eta_C\chi. \tag{51}$$

Next we turn our attention to the normal stress continuity at $r=\rho_D$, which takes the form

$$\nu p_C(\rho_D^+)-2\nu\eta_C u'(\rho_D^+)+(1-\nu)p_E(\rho_D) = p_E(\rho_D), \tag{52}$$

that is

$$p_C(\rho_D^+) = p_E(\rho_D)+2\eta_C u'(\rho_D^+), \tag{53}$$

finally leading, for $p_{gel}=0$, to

$$\begin{aligned} 2\gamma = \quad & \frac{1}{\nu(1-\nu)}\frac{\chi R^3}{3K}\left\{\frac{R}{\rho_D}\left[1-\left(\frac{\rho_P}{R}\right)^3\right]-\frac{3}{2}\left[1-\left(\frac{\rho_P}{R}\right)^2\right]\right\} \\ + \quad & \frac{4}{3}\eta_C\chi R\left(\frac{R}{\rho_D}\right)^3\left[1-\left(\frac{\rho_P}{R}\right)^3\right]. \end{aligned} \tag{54}$$

The study of (54) has been performed in [29] (actually a slightly different expression was used there, corresponding to a simplified definition of $\hat{p}$). The right hand side of (54) is a function of R tending to infinity both for $R\to\infty$ and in correspondence of the critical value of R for which $\rho_D\to 0$ and below which the interface $r=\rho_D$ is not defined. There is only one minimum, that we may call $2\gamma^*$, which defines a critical value of the surface tension, discriminating between existence and non-existence of a steady state. Thus the problem of finding R is solvable if and only if $\gamma>\gamma^*$. In [29] we found that a value slightly greater than 0.01 dyne/cm for γ is compatible with our reference situation with $R=1$ mm. The value of γ increases to about 0.05 dyne/cm, if K is reduced to $10^{-8}\,\mathrm{cm}^3\,\mathrm{sec/g}$. Clearly when $\gamma>\gamma^*$ equation (54) has two solutions. Since the spheroid grows to a steady state from a small initial size, we can say that the physical solution is the smaller.

Remark 5.5. *In the liquid-dominated case (see Remark 5.4) the term in (54) containing viscosity can be neglected. In such a case the solution is going to depend just on the product $K\gamma$.*

Remark 5.6. *Equation (54) emphasizes that, keeping γ fixed, equilibrium becomes impossible when η_C is raised above some threshold. This fact has a physical interpretation. Indeed a steady state configuration requires that all cells possess a radial inward directed velocity. If viscosity is too large, the inward motion is hindered and the spheroid tends to grow indefinitely to the exterior.*

Remark 5.7. *By recalculating the values of w_P corresponding to the solutions predicted by (54) for different values of the oxygen concentration σ^*, w_P is actually found to vary. So the conjecture of the energy based approach that the power w_P can be defined as a characteristic cell parameter is disconfirmed. Nevertheless, in the simulation of [29] the variation of w_P was only of the order of* 15%.

6. Concluding Remarks

It is well known that multicellular spheroids in an advanced stage of their evolution contain a considerable fraction of dead cells and debris, mostly concentrated in the central region. It is therefore quite natural to speak of a necrotic core. Describing the structure of that region, as it results from cells degradation, turns out to be a crucial step in modelling the whole system, since, irrespectively of the constitutive equations selected for the various components, the growth of the spheroid will depend on how the viable region interacts mechanically with the necrotic core. Various schemes have been proposed in the literature since the early paper by Greenspan [34], ranging between two extremes: from a completely solid to a completely liquid core. The necrotic zone is frequently described as a region bounded by a sharp interface. Clearly, the presence of interfaces within a spheroid separating cells in different states is an extrapolation which is frequently adopted (the spherical symmetry itself is an idealization). In our opinion the sharp boundary approach is quite sensible, since it simplifies the conceptual geometrical scheme without deeply altering the actual cell distribution.

In this paper we have dedicated some attention to the modelling of the necrotic zone, confining ourselves to the analysis of the steady state (when it exists). We started with a short summary of the relevant literature, trying to point out which specific assumption in each of the considered models (most of the times related with the necrotic region) guarantees the existence of a steady state. After having illustrated the implications of assuming a completely solid necrotic core, we review some theory developed in the papers [28, 29] in which the necrotic region consists of a solid shell encasing a liquid nucleus. We took this opportunity for carrying out a critical analysis not only of the approach of those papers, in which we have adopted a two-fluid scheme, but also of the general conceptual difficulties accompanying such a representation of the spheroids mechanics. We have emphasized that there are good motivations for selecting a relatively simple model, since not only the number of parameters to be determined increases with the complexity of the mathematical scheme, but also the uncertainty of their identification becomes more and more serious. On the other hand, there is a price to pay for simplicity, since one cannot expect that models with few parameters can give a particularly accurate description of intrinsically complex systems. Thus, as a general rule in mathematical biology, the focal point in modelling is to find a reasonable compromise. Of course it is only the comparison with experimental data which can say to what extent the compromise is acceptable.

Under this respect we found that the approach followed in [28], based on energy balance, and the one used in [29], imposing the continuity of normal stress throughout the system, perform in a comparable way, despite the fact that - strictly speaking - they are not mutually compatible. Indeed, while the principle of normal stress continuity always applies, the energy balance proposed in [28] involved the conjecture that all proliferating cells deliver the same amount w_P of mechanical power. Such a conjecture is not confirmed by the results of [29]. This is an indication that defining a quantity like w_P may not be correct. As usual, one has to be cautious in stating what is or is not correct, since it has to be considered that all these results have been obtained in a framework of a model that we already know to be largely approximated. In the same spirit we cannot even refuse the third (quite appealing) option that equilibrium is characterized as the configuration minimizing the dissipation of mechanical power, which does not appear to be met by any of the previous models.

That said, once it is made clear that we cannot ask too much to a mechanically simple model, the studies performed both in [28] and in [29] lead to results that are quantitatively acceptable and qualitatively interesting, since they permit to ascertain the influence of the basic parameters on the possible attainment of equilibrium, and on the size eventually reached by the spheroid under specified environmental conditions. A quantity which may play a critical role is the so called tumour surface tension, which in some of the models reviewed is necessary for equilibrium to be attained. This is indeed the case of the model in [29].

A step further, already envisaged in [29] on the wake of other papers (e.g. [2]), consists in modelling the cell component as a Bingham fluid, thus possessing a yield stress acting as a threshold to allow deformations (and hence the radial flow typical of cells in a spheroid). The reason to shift to a Bingham flow is to better represent the effect of intercellular bonds that can bear some limit tension and have to be broken to allow deformations, a responsibility that is totally assigned to viscosity in the Newtonian

framework. We find the Bingham approach particularly stimulating and we plan to study the evolution of "Bingham spheroids" in a future paper. We may anticipate that the selection of a Bingham-like constitutive law is a delicate issue, owing to the peculiar feature of the "fluid" considered, which is actually incompressible, but in which volume is not preserved due to proliferation.

As a general conclusion we may say that the scheme in which the necrotic core has an interface separating a liquid nucleus from a solid shell allows to describe the complex radial flow within a spheroid at equilibrium, either in a fully Newtonian framework, or adopting a Bingham scheme for the fluid representing cells. Despite all the limitations accompanying the two-fluid models, that we have carefully pointed out, the results obtained are meaningful and it makes sense to extend the whole analysis to the evolutionary case.

Possible extensions of the model may include other aspects that are biologically important, among them the cell inhibition by contact. In the review section of this paper we have mentioned models which include inhibitors of proliferation generated by dead cells disaggregation. A possibly more important cause of inhibition is related with crowding. Since the cell volume fraction does not vary much within the spheroid, crowding can be sensed via the stress. For instance in the Newtonian scheme cell proliferation can be assumed to stop when p_C exceeds some threshold.

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