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Residual stress generation and necrosis formation in multi-cell tumour spheroids

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Abstract. We consider how cell proliferation and death generate residual stresses within a multi-cell tumour spheroid (MCTS). Previous work by Jones and co-workers [8] has shown that isotropic growth in a purely elastic MCTS produces growth induced stresses which eventually become unbounded, and hence are physically unrealistic. Since viscoelastic materials show stress relaxation under a fixed deformation we consider the effect of the addition of a small amount of viscosity to the elastic system by examining formation of equilibrium stress profiles within a Maxwell type viscoelastic MCTS. A model of necrosis formation based upon that proposed by Please and co-workers (see [16] [17] [18]) is then presented in which necrosis forms under conditions of adverse mechanical stress rather than in regions of extreme chemical stress as is usually assumed. The influence of rheology on necrosis formation is then investigated, and it is shown that the excessive stress generated in the purely elastic tumour can be relieved either by the addition of some viscosity to the system or by accounting for an inner necrotic interface with an appropriate stress boundary condition.

1. Introduction

In the early stages of development, an *in vivo* tumour may have no vascular structure of its own and consequently must rely on diffusion for supply of vital nutrients and removal of waste products. Since the cells in the tumour consume nutrient in order to proliferate this naturally leads to the formation of nutrient deficient regions sufficiently far from the nutrient source. As the outer free surface of the tumour moves in response to cell proliferation its central regions may become more and more nutrient deficient until eventually the interior of the tumour may become so lacking that cells can no longer survive, and they die. Such cell death is known as necrotic, and an accumulation of the debris expelled after such death is commonly called a necrotic region. As a means of modelling avascular growth *in vitro*, the multi-cell tumour spheroid (MCTS) has proven to provide a structurally relevant model of the *in vivo* situation [11]. In such spheroids a central necrotic region is commonly seen to appear once the spheroid outer radius reaches a critical size of around 500μm [10]. Additionally, a well defined spheroid architecture is often observed whereby the MCTS grows with an outer shell of viable cells, around 100-300μm thick [10],

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consisting of a rim of proliferating cells close to the spheroid outer surface surrounding a region of viable but quiescent cells which in turn surround the necrotic core.

The traditional view of necrosis formation is therefore based upon deficiency of energy providing chemicals such as oxygen, or glucose and/or accumulation of harmful metabolic byproducts and associated pH changes (although this is known to be an incomplete picture [13]). For this reason, mathematical models have also focused on necrosis formation by nutrient deficiency alone (see for example [24] [25]). In these models the spheroid outer surface is taken to move in response to net proliferation within the tumour and its position at any time can be determined from cell and nutrient conservation considerations alone. If a necrotic core is established at a critical concentration of a limiting nutrient then the position of the necrotic interface can also be determined by conservation of cells, and nutrient. In this case the specific rheology of the tumour is incidental to the determination of the positions of the necrotic and free outer surfaces of the tumour, and need not be considered by the model. However, in practice, the specific rheology of the tumour, and its surrounding medium do appear to play a crucial role in tumour development. For example, a recent report by Helmlinger and co-workers [6] focused on the influence of growth induced stress in the tumour surroundings on spheroid development. By culturing spheroids in gels of different stiffnesses they showed that increasing the medium stiffness reduced the rate of tumour growth and equilibrium size. Furthermore, Chen and co-workers [3] have used the experimental data of Helmlinger et al. in a mathematical investigation of the effects of solid stress on spheroid development, including the effect on necrosis formation. Their model predicts the behaviour outlined by Helmlinger et al. as well as suggesting that increasing the surrounding gel stiffness may delay the onset of necrosis. The relationship between gel stiffness and necrosis formation has yet to be determined experimentally, however the work by Chen et al. and Helmlinger et al. does highlight the importance of growth induced residual stress on spheroid growth rate and evolution of architecture. In particular, these results emphasise the interaction between cell proliferation and tissue rheology and suggest that modelling of MCTS growth may benefit from further consideration of the forces generated within the tumour by cell proliferation.

In order to proliferate a cell will, in general, take up nutrients and water, expand and then divide in two daughter cells. Conversely, when a cell suffers a necrotic death its membrane ruptures and its contents disperse. In a tissue, where a population of cells are proliferating and dying, this leads to the generation of a stress field associated with the local rate of net proliferation. Intuitively, we may expect that in those areas where nutrient levels and proliferation rates are high, cells will be in compression, with the compressive stress being transmitted to the cells through the extra-cellular matrix (ECM). Conversely, in regions where nutrient levels are low, and cell death is predominant, cells may be expected to be in tension. This intuitive link between proliferation rate and stress suggests that an investigation of necrosis formation in terms of adverse mechanical stress rather than in terms of adverse *chemical* stress may prove fruitful. In a number of recent papers, Please and co-workers have explored the concept of stress dependent necrosis (for example, see [16] [18]). By considering a tumour consisting of two inviscid phases —cells

and ECM forming one phase and extracellular water forming a second —they produced a model of cellular proliferation by inter-phase mass exchange in which necrosis is allowed to form when the local inter-cellular pressure falls below the local extra-cellular water pressure. In such a model, cells are assumed to be unable to sustain any significant tension, so that when the extra-cellular water pressure becomes higher than the local inter-cellular pressure they are ripped from each another and rupture. Within this modelling framework, necrosis formation is therefore explicitly coupled to tissue rheology, and in this paper we will pursue these ideas further.

If the population of cells is large enough it is appropriate to assume that they form a continua and to consider properties of *volume elements* rather than individual cells. Since techniques in continuum mechanics are well developed this approach has proved successful in modelling many biological phenomena involving living tissues (see for example [2] [14] [15]). In situations where tissue growth is an important consideration, models have traditionally focused on multi-phase modelling, incorporating growth of one phase by mass transfer from the other phases (for example, see [16]). However, growth can also be modelled as the swelling of a single phase. If the growth rate over the timescale of interest is large enough, the inclusion of growth induced stresses must be accounted for since they may be the dominant forces acting on the tissue. Consequently, a number of authors have adapted the traditional derivations of the equations of continuum mechanics to allow a single-phase material to be simultaneously subject to external forces and internal growth-induced stresses (see for example, [4] [12] [19] [21] [22]). When considering volumetric growth (where growth is continuously distributed throughout the whole body) this involves adapting the stress-strain relationship of the material in question *and* the conservation equations. Generally, the approach is to allow infinitesimal volume elements to grow individually from a continuous stress free reference state to a second discontinuous stress free reference state. The "grown" infinitesimal elements are then reassembled to form a final continuous stressed state. If no remoulding stress is required to keep the body intact then the growth is described as compatible, whereas if a remoulding stress is needed to ensure continuity then the growth is known as incompatible. The remoulding stress required to maintain continuity is known as the residual stress, and the generation of residual stress in many biological tissues, including tumour spheroids has been observed [23]. One approach by Jones et al. in [8] uses an analogy with thermal expansion to derive a constitutive relationship for a growing elastic material. This is then used to generate time-dependent residual stress distributions within tumours of various geometries. In particular they show that in a tumour growing in a spherically symmetric way the growth induced stress eventually becomes unbounded. To avoid this unphysical situation they suggest exploration of a multi-phase model consisting of live tumour cells, dead tumour cells and extra-cellular water. In this paper we adopt an alternative approach based on the observation that some soft biological tissues exhibit stress relaxation under a constant load [7], and therefore consider stress generation in a single phase viscoelastic tumour.

The structure of the remainder of this paper is as follows: first, we outline a mathematical model for growth in viscoelastic materials which will then be used to

look at the generation of residual stresses within a tumour in an arbitrary geometry. We will be particularly concerned with how to appropriately introduce viscous effects into a model similar to that presented by Jones et al. [8]. We then look specifically at stress generation within a spherically symmetric tumour. Subsequently, we consider necrosis formation in terms of mechanical stress conditions in a manner similar to that proposed by Please et al. in [16]. An appropriate *thin shell* asymptotic approximation is then discussed and time-dependent asymptotic approximations to the stress are derived in this limit. The effect of rheology on necrosis formation is then investigated and parameter regimes in which necrosis can form are outlined. In particular we describe how steady state stress distributions can be achieved within a steady state size tumour, and thus how the unbounded stresses found by Jones et al. in [8] can be relieved.

2. Mathematical Model

Consider growth of an avascular tumour bathed in a nutrient supplying medium. The nutrient, which, for example, may be thought of as oxygen or glucose, diffuses throughout the tumour and is consumed by the cells. Making the assumption that the cellular proliferation rate is determined solely by the available local nutrient concentration (this assumption is reasonable, although some recent reports have suggested that stress-dependent cell proliferation may also be important in tumour development —see [1] for example) the resulting nutrient gradient produces a non-uniform cellular proliferation rate which generates a stress distribution with compressive stress in nutrient rich regions and tensile stress in nutrient deficient regions.

We denote the tumour cell number density per unit volume by $n(\mathbf{x}, t)$, nutrient concentration per unit volume by $c(\mathbf{x}, t)$, tumour cell velocity by $\mathbf{v}(\mathbf{x}, t)$, and the stress tensor of cellular material (including extra-cellular matrix) by $\boldsymbol{\sigma}(\mathbf{x}, t)$. Equations describing evolution of these variables are discussed now. We assume that nutrient is transported by Fickian diffusion with a constant diffusion coefficient, D_c, and is consumed by the cells at a rate proportional to both the cell number density, and the nutrient density. Since the timescale for evolution of nutrient (minutes) is significantly less than that of the doubling time of the tumour mass (hours) we assume that nutrient evolves in a quasi-steady way (see [8] [24] and others) and that

$$0 = D_c \nabla^2 c - Acn, \tag{1}$$

where A is a constant of proportionality.

Assuming that cells do not actively migrate and have a nutrient dependent net proliferation rate $F(c)$, then cell mass conservation is given by

$$\frac{\partial n}{\partial t} + \nabla \cdot (\mathbf{v}\, n) = nF(c). \tag{2}$$

Within the physiological range of deformation, most soft tissues can be regarded as incompressible [7]. Making this assumption allows (2) to be reduced to

$$\nabla \cdot \mathbf{v} = F(c), \tag{3}$$

and (1) becomes

$$0 = D_c \nabla^2 c - A_* c, \tag{4}$$

where the cell packing density is constant, $n = n_0$, throughout the tumour and $A_* = An_0$.

The movement of the free outer surface of the tumour can be determined from the cell velocity by imposing a kinematic condition on the free surface which designates that the surface moves with the velocity of the cells of which it is constituted. Thus, evolution of the free outer surface, $g(\mathbf{x}, t) = 0$, is given by

$$\frac{\partial g}{\partial t} = \mathbf{v} \cdot \mathbf{n}, \tag{5}$$

where $\mathbf{n}$ is the unit outward normal to the free surface.

In order to determine the generation of residual stress within the MCTS a stress-strain relationship must be prescribed which accurately models the rheological properties of the tissue in question and appropriately includes growth terms. A number of authors have discussed the rheological properties of soft biological tissue. For example, Holzapfel [7] outlines the bio-mechanics of soft tissue such as tendon, ligament, skin and cartilage. In general such tissues behave as non-homogeneous, anisotropic, non-linear materials, with some specifically showing viscoelastic behaviour (such as creep and stress relaxation). Additionally, if subjected to loads beyond physiological range they will, in general, deform permanently. Consequently, construction of an accurate constitutive relationship for soft tissues is exceptionally complex and tissue specific. Therefore, in order to make some progress, we will impose a number of simplifications. Firstly, since we will consider only growth in a small spherical monoculture we will assume that the soft tissue under consideration is homogeneous, and isotropic. Additionally, since the viscoelastic nature of soft tissues is associated with shear interactions between components of the ECM (in particular collagen and the proteoglycan network) [7] and because collagen behaves as a single relaxation time Maxwell fluid [9] we shall introduce viscosity in this manner. We perceive that by describing the tissue as a single relaxation time Maxwell fluid much of the complex behaviour of the tissue (anisotropy, inhomogeneity etc.) is ignored. However, importantly, this assumption does provide a simple and feasible way to introduce viscoelasticity into the model as well as allowing good comparison of our model with the one presented by Jones et al. in [8].

Growth is incorporated into the stress strain relationship by decomposing total strain rate into a material strain rate and a growth strain rate, and formulating the stress tensor in terms of the material strain rate only. Hence, the stress tensor for an isotropically growing incompressible Maxwell fluid is given by

$$\boldsymbol{\sigma} = -P\,\boldsymbol{\delta} + \boldsymbol{\tau}, \tag{6}$$

$$\gamma \frac{\delta \boldsymbol{\tau}}{\delta t} + \boldsymbol{\tau} = 2\mu(\mathbf{D} - \mathbf{D}^G), \tag{7}$$

where P is the pressure, $\boldsymbol{\tau}$ is the trace-free deviatoric stress, $\mathbf{D}$ is the rate of strain tensor,

$$\mathbf{D} = \frac{1}{2}\left[\frac{\partial v_i}{\partial x_j} + \frac{\partial v_j}{\partial x_i}\right], \tag{8}$$

$\delta/\delta t$ is an appropriate tensorial time derivative, $\gamma = G/\mu$ the tissue relaxation time (where G is the shear elastic modulus, and μ is the viscosity) and $\mathbf{D}^G$ is the rate of growth tensor. Prescription of the rate of growth tensor is made from biological considerations, however if the material is incompressible then (3) imposes the condition $\mathrm{Tr}(\mathbf{D}) = F(c)$. If, in addition, growth is isotropic then

$$\mathbf{D}^G = \frac{1}{3}F(c)\boldsymbol{\delta}. \tag{9}$$

In order to maintain tensorial character, the derivative used on the left hand side of (7) must be objective and follow the material elements. Since such derivatives are non-unique, one must be chosen, and in many respects the choice of derivative is arbitrary. Therefore, following Jones et al. [8], we chose the Jaumann or co-rotational derivative which advects and rotates with the instantaneous angular velocity of the fluid. Thus the constitutive relationship for an incompressible co-rotational Maxwell material growing isotropically can be expressed as

$$\gamma\left[\frac{D\boldsymbol{\tau}}{Dt} - \mathbf{W}\boldsymbol{\tau} + \boldsymbol{\tau}\mathbf{W}\right] + \boldsymbol{\tau} = 2\mu\left[\mathbf{D} - \frac{1}{3}\nabla\cdot\mathbf{v}\,\boldsymbol{\delta}\right], \tag{10}$$

where $\mathbf{W}$ is the vorticity tensor.

In order to find the residual stress generated by continuous growth within a body, the equilibrium stress equation must be solved (see [19]). In the absence of any body forces, the stress therefore satisfies

$$\nabla\cdot\boldsymbol{\sigma} = 0. \tag{11}$$

Equations (3), (4), (5), (6), (10) and (11) along with appropriate boundary and initial conditions comprise our model.

2.1. The onset of necrosis

When $\gamma = 0$ equations (6) and (10) describe the constitutive relation for continuous isotropic growth in a Newtonian viscous fluid. In this case, by using (11), the pressure can be shown to satisfy

$$\nabla P = \nabla^2\mathbf{v} + \frac{1}{3}\nabla(\nabla\cdot\mathbf{v}), \tag{12}$$

$$= \frac{4}{3}\nabla(\nabla\cdot\mathbf{v}) - \nabla\times(\nabla\times\mathbf{v}). \tag{13}$$

In situations where $\nabla\times\mathbf{v} = 0$ (for example the spherically symmetric case) equation (13) shows that the pressure is related linearly to the rate of dilation. Consequently, in a purely viscous fluid, conditions for necrosis formation can be formulated in

terms of the net stress (which is proportional to the pressure) rather than in terms of the local rate of proliferation. By analogy with this example, for the remainder of this paper we will assume that appropriate conditions for formation of a necrotic region can also be given in terms of the net stress for *any* material. Therefore, by comparison with the model for necrosis formation given by Please et al. in [16], we will assume that necrosis forms once the net stress between cells becomes tensile and consequently that necrosis forms when $\mathrm{Tr}(\boldsymbol{\sigma}) = 0$. Since, in equation (6), we partitioned the stress into a pressure and a trace-free deviatoric stress this means that necrosis will form once the inter-cellular pressure, $P = 0$. In making this assumption we are inferring that cells cannot sustain a significant tension and under such circumstances will die.

2.2. *Growth of a spherically symmetric tumour*

Please and Landman [18] use a simple model for cell proliferation and necrotic death by defining a positive constant cell proliferation rate in regions where the nutrient concentration is above a critical value $c = \alpha$, and a negative constant proliferation rate for $c \leq \alpha$. We follow their example and set

$$F(c) = \begin{cases} a & c > \alpha, \\ -a\lambda & c \leq \alpha, \end{cases} \tag{14}$$

where a is the constant growth rate in the proliferating region and λ describes the relative death rate in the tumour interior.

Since we consider growth of a spherically symmetric tumour, we will work in spherical polar co-ordinates. The equations of motion are then nondimensionalised by assuming that the nutrient concentration in the surrounding medium is constant, c_0. The following scalings are used:

$$c = c_0\widehat{c}, \qquad \mathbf{x} = \sqrt{\frac{D_c}{A_*}}\widehat{\mathbf{x}}, \qquad \mathbf{v} = a\sqrt{\frac{D_c}{A_*}}\widehat{\mathbf{v}},$$
$$t = \frac{1}{a}\widehat{t}, \qquad \boldsymbol{\sigma} = G\widehat{\boldsymbol{\sigma}}.$$

In the spherically symmetric case, off diagonal elements of the stress tensor are zero, and denoting $\boldsymbol{\tau} = \mathrm{diag}(T, T_\theta, T_\phi)$, the stress tensor has the form,

$$\begin{aligned} \boldsymbol{\sigma} &= \begin{pmatrix} \sigma_r & 0 & 0 \\ 0 & \sigma_\theta & 0 \\ 0 & 0 & \sigma_\phi \end{pmatrix} \\ &= \begin{pmatrix} -P + T & 0 & 0 \\ 0 & -P + T_\theta & 0 \\ 0 & 0 & -P + T_\phi \end{pmatrix}, \end{aligned} \tag{15}$$

where we have introduced the notation $P = P(r, t)$, $T = T(r, t)$, $T_\theta = T_\theta(r, t)$, $T_\phi = T_\phi(r, t)$ for simplicity, and, in this case, $c = c(r, t)$ and $\mathbf{v} = [v(r, t), 0, 0]$. Upon dropping hats for simplicity, the nondimensional equations of motion are

$$\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c}{\partial r}\right) = c, \tag{16}$$

$$\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 v\right) = F(c), \tag{17}$$

$$\frac{\partial T}{\partial r} + 3\frac{T}{r} = \frac{\partial P}{\partial r}, \tag{18}$$

$$2\left[\frac{\partial v}{\partial r} - \frac{1}{3}F(c)\right] = \frac{\partial T}{\partial t} + v\frac{\partial T}{\partial r} + \delta T, \tag{19}$$

$$T + T_\theta + T_\phi = 0, \tag{20}$$

$$T_\theta = T_\phi. \tag{21}$$

Where $\delta = \gamma/a$ represents the ratio of the elastic response of the viscoelastic fluid to the viscous response over the timescale of interest and the nondimensional rate of dilation is given by

$$F(c) = \begin{cases} 1 & c > \alpha, \\ -\lambda & c \leq \alpha. \end{cases} \tag{22}$$

At some critical time, the interior of the tumour may fall into tension and necrotic region will form. When this happens, the cell number density in the necrotic core may vary, allowing for gradual transition to complete necrosis. However, before this occurs, the cellular material in the tumour will remain fully packed and incompressible. Boundary conditions as $r \to 0$ and on the outer surface, $r = R(t)$, must be provided to properly close the problem. As $r \to 0$ we impose the conditions

$$\frac{\partial c}{\partial x} = 0, \qquad v = 0, \tag{23}$$

allowing no flux of nutrient or cells at this point in order to preserve symmetry. On the free outer surface, $r = R(t)$, we impose the kinematic condition (5),

$$\frac{dR}{dt} = v(R, t) \qquad \text{with} \qquad R(0) = R_0. \tag{24}$$

It has been observed that the outer surface of many tumour spheroids are remarkably smooth [20] suggesting that cells on the outer surface, where nutrient levels are high may be able to sustain a certain level of tension due to their increased ability to adhere to each other via Cadherin attachments. However, away from the free surface, where nutrient is not so available and conditions are more severe, this ability of cells to sustain some tension is diminished [18]. This adherence on the free surface will produce a pressure difference proportional to the radius of curvature of the surface and can be thought of as a surface tension, as suggested in [5]. Therefore on $r = R(t)$ we also impose the condition

$$\sigma_r(R, t) = -\frac{2\Gamma}{R}, \tag{25}$$

with the minus sign introduced to give a compressive stress in the radial direction, and Γ being the surface tension coefficient. In order to complete the boundary conditions we assume that nutrient concentration is continuous over the outer surface,

such that $c(R, t) = 1$. Since equation (10) requires an initial condition, we additionally impose the condition $T(r, 0) = 0$ to ensure that the tumour evolves from a stress free reference state.

The above model holds until, at some point in the tumour, $P = 0$ and a necrotic region forms (this condition occurs first at the centre due to symmetry). Once there is a necrotic region, the equations of motion must be solved subject to conditions on the necrotic interface rather than those given in (23). The appropriate form of these conditions remains uncertain, although some can be derived by analogy to those presented by Please et al. in [16]. However, for the purposes of this paper, since we are concerned with finding *when* necrosis will form and not the subsequent development, we do not need to specify them here.

3. Solutions

Integrating (16) gives

$$c = \frac{R \sinh(r)}{r \sinh(R)}. \tag{26}$$

For most tumours it is appropriate to consider R large. In this case, sufficiently near the surface, (26) can be approximated by $c \sim \mathrm{e}^{r-R}$. Since the nutrient concentration is only exponentially small away from this region, this forms a reasonable approximation to the nutrient concentration throughout the whole tumour. From this approximation it follows that the critical radius at which the critical nutrient concentration, α, is reached is approximated by $r_c = R + \ln(\alpha)$.

The cell velocity can be found by solving (17) subject to the condition given in (23) and by patching the solutions together at the interface $r = r_c$. Doing so gives

$$v = \begin{cases} -\frac{\lambda r}{3}, & 0 \le r \le r_c, \\ \frac{1}{3r^2}(r^3 - (1+\lambda) r_c^3), & r_c \le r \le R. \end{cases} \tag{27}$$

This velocity profile is then used to solve (19) in each of the two regions either side of r_c and the pressure is then determined from (18). In order to solve (19) we use the method of characteristics.

We will denote the region $r_c \le r \le R$ as the proliferating region (since there is net cell proliferation there) and the region $0 \le r \le r_c$ as the quiescent region (since there is net cell death there). Characteristic curves which start in the proliferating region must patch onto characteristic curves in the quiescent region at $r = r_c$ to ensure continuity of stress across this interface. For this reason, the quiescent region can be split into two separate sub-regions. In one sub-region, solutions must be calculated along characteristics which start at $r = r_c$ with a patching condition from the proliferating region. In the other sub-region, solutions must be calculated along characteristics which start in the quiescent region with initial conditions at $t = 0$. We will call the sub-region bounded below by the origin and above by the last characteristic to *start* in the quiescent region (the curve $r = r_c \mathrm{e}^{-\lambda t/3}$) the *core* region and the region bounded below by this characteristic and above by $r = r_c$ the *central* region. The core region outlined above is analogous to the core region

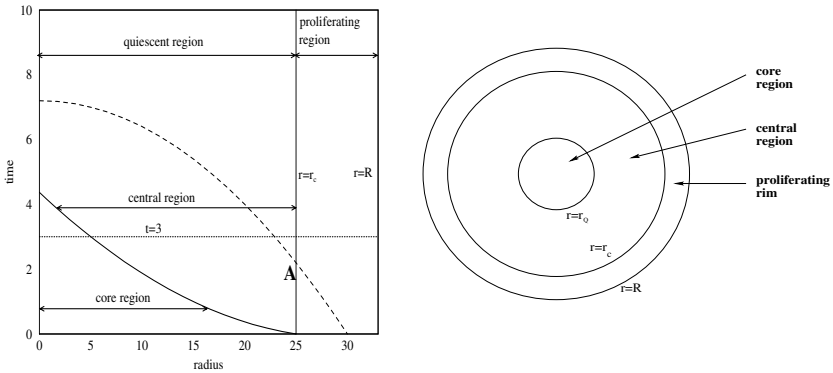

Fig. 1. Illustration of the three regions in a steady state size tumour for varying time (*left*). The core-central interface is given by the bold curve starting at $t = 0$ from $r = r_c$. The dashed line illustrates a typical characteristic starting in the proliferating region. At the point **A** this characteristic leaves the proliferating region and enters the central region and a patching condition is imposed. The corresponding spatial regions in a tumour for a representative time $t = 3$ are given on the *right*.

presented by Jones et al. in [8] and corresponds to those cells initially present in the tumour which have never proliferated. Thus, stresses will be found in *three* (proliferating, central and core) regions. Figure 1 illustrates these three regions in a tumour of fixed size. In this figure, the illustration on the left shows the three time-dependent regions of the tumour. The core-central interface is given by the bold curve starting at $t = 0$ from $r = r_c$. The dashed line illustrates a typical characteristic starting in the proliferating region. At the point **A** this characteristic leaves the proliferating region and enters the central region and a patching condition must be imposed in order to ensure continuity of stress. At any time the position of the core-central interface is denoted by $r = r_Q$. For a representative time of $t = 3$ the positions of the three interfaces are illustrated in the figure on the right.

The modelling presented so far allows for formation of a tumour with a rim of proliferating cells surrounding a central quiescent region. This proliferating region has thickness $-\ln(\alpha)$, which, since $0 \leq \alpha \leq 1$, allows for a proliferating shell of any thickness. However, it is commonly observed that this rim is thin with respect to the tumour outer radius [11]. Therefore, in order to allow analytic solutions to be obtained a thin shell limit will be imposed *a priori*, by assuming that $\ln(\alpha)/R$ is small.

It is also commonly seen that a MCTS will grow to a steady state fixed size at which the rate of cell proliferation near the tumour periphery balances the rate of cell loss in the interior. Since our main emphasis is on the existence of a steady state stress distribution rather than its detailed time evolution, in keeping with the analysis of Jones et al. [8], we present evolving stress distributions within a steady state size non-necrotic tumour. From equations (24) and (27), a steady state size tumour has radius $R = R_*$ given by

$$R_* = \frac{(1+\lambda)^{1/3}\ln(\alpha)}{1-(1+\lambda)^{1/3}}. \tag{28}$$

Consequently, for a tumour at its steady state size, the thin shell approximation is equivalent taking the limit $\lambda \to 0$.

3.1. Stress distribution within a steady state sized tumour

Letting superscript symbols P, Q and C indicate solutions in the proliferating ($r_c \le r \le R_*$), central ($r_c e^{-\lambda t/3} \le r \le r_c$) and core ($0 \le r \le r_c e^{-\lambda t/3}$) regions respectively, the leading order asymptotic approximations to the stresses in the radial direction as $\lambda \to 0$ are found to be

$$\sigma_r^P \sim \frac{4}{\delta}(1 - e^{-\delta t}) \ln\left(\frac{R_*}{r}\right) - \frac{2\Gamma}{R_*}, \tag{29}$$

$$\sigma_r^Q \sim \frac{4}{\delta}\left\{(1 - e^{-\delta t}) \ln\left(\frac{R_*}{r_c}\right) + \frac{\lambda}{3\delta}\left[1 - \left(\frac{r}{r_c}\right)^{3\delta/\lambda}\right] + e^{-\delta t} \ln\left(\frac{r}{r_c}\right)\right\} - \frac{2\Gamma}{R_*}, \tag{30}$$

$$\sigma_r^C \sim \frac{4}{3\delta}\left\{3(1 - e^{-\delta t})\left[\ln\left(\frac{R_*}{r_c}\right) + \frac{\lambda}{3\delta}\right] - \lambda t e^{-\delta t}\right\} - \frac{2\Gamma}{R_*}, \tag{31}$$

where each of these solutions retains an $\mathcal{O}(\lambda)$ error. These results show that a steady state stress distribution in the radial direction can be achieved for all $\delta \neq 0$.

Figure 2 illustrates the evolution of stress in the radial direction within a steady state size tumour for parameter values $\lambda = 0.0375$, $\Gamma = 1$, $\alpha = 0.7$ and $\delta = 0.1$ (these parameter values give a steady state outer radius of $R_* = 29.63$ to allow comparison with those solutions presented by Jones et al. in [8]).

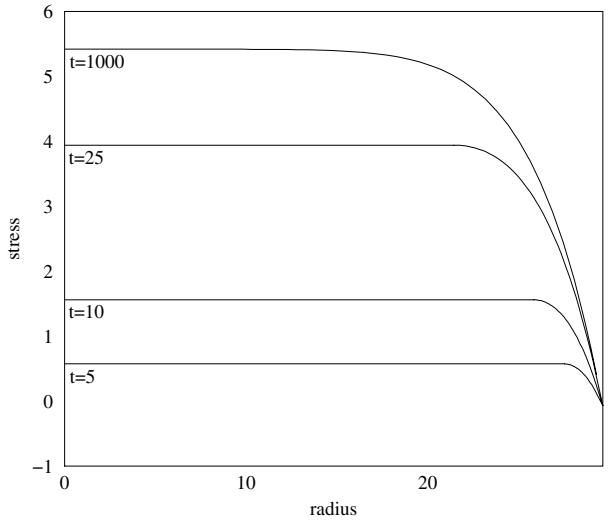

Fig. 2. Example of stress evolution in the radial direction in a steady state size tumour with thin proliferating shell. Parameter values: $\lambda = 0.0375$, $\Gamma = 1$, $\alpha = 0.7$ and $\delta = 0.1$.

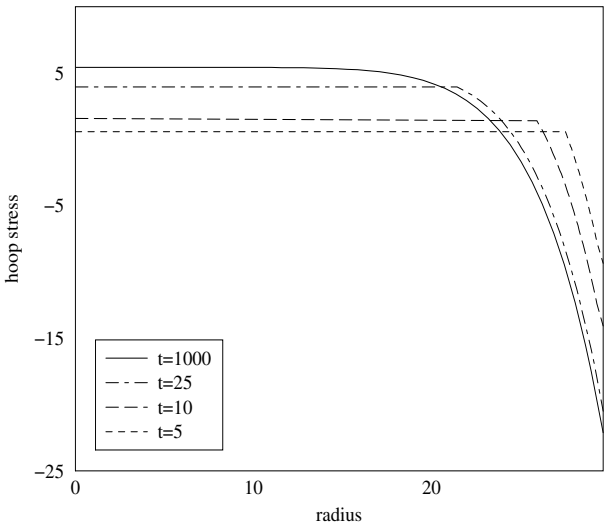

Fig. 3. Example of hoop stress evolution in a steady state size tumour with thin proliferating shell. Parameter values as in figure 2.

Hoop stresses can also be approximated in the three regions, since $\sigma_\theta = \sigma_\phi = \sigma_r - 3T/2$. Calculations show that

$$\sigma_\theta^P \sim \frac{4}{\delta}(1 - e^{-\delta t})\left[\ln\left(\frac{R_*}{r}\right) - \frac{1}{2}\right] - \frac{2\Gamma}{R_*}, \tag{32}$$

$$\sigma_\theta^Q \sim \frac{4}{\delta}\left\{(1 - e^{-\delta t})\ln\left(\frac{R_*}{r_c}\right) + \frac{\lambda}{3\delta}\left[1 - \left(\frac{r}{r_c}\right)^{3\delta/\lambda}\right] + e^{-\delta t}\left[\ln\left(\frac{r}{r_c}\right) + \frac{1}{2}\right]\right\} - \frac{2\Gamma}{R_*}, \tag{33}$$

and $\sigma_\theta^C = \sigma_r^C$.

Figure 3 shows evolution of hoop stresses within a steady state size tumour, for parameter values as in figure 2. Again an equilibrium stress distribution can be achieved for all $\delta \neq 0$.

Using the same notation, the pressures in the various regions are found to be

$$P^P \sim \frac{4}{3\delta}(1 - e^{-\delta t})\left[1 - 3\ln\left(\frac{R_*}{r}\right)\right] + \frac{2\Gamma}{R_*} \tag{34}$$

$$P^Q \sim \frac{4}{3\delta}\left\{\left(\frac{r}{r_c}\right)^{3\delta/\lambda} - e^{-\delta t} - 3(1 - e^{-\delta t})\ln\left(\frac{R_*}{r_c}\right) - \frac{\lambda}{\delta}\left[1 - \left(\frac{r}{r_c}\right)^{3\delta/\lambda}\right] - 3e^{-\delta t}\ln\left(\frac{r}{r_c}\right)\right\} + \frac{2\Gamma}{R_*}, \tag{35}$$

$$P^C \sim \frac{4}{3\delta}\left\{\lambda t e^{-\delta t} - 3(1 - e^{-\delta t})\left[\ln\left(\frac{R_*}{r_c}\right) + \frac{\lambda}{3\delta}\right]\right\} + \frac{2\Gamma}{R_*}. \tag{36}$$

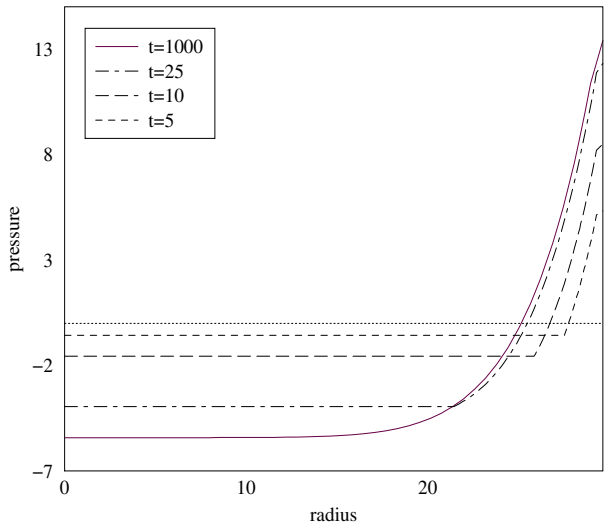

Fig. 4. Example of pressure evolution in steady state size tumour with thin proliferating shell. Parameter values as in figures 2 and 3. It can be seen that in the interior P becomes negative almost immediately and hence it is expected that necrosis will have formed well before the non-necrotic steady state pressure distribution can be achieved.

Figure 4 illustrates pressure evolution within a steady state size tumour for parameter values as in figures 2 and 3.

Although a steady state stress distribution does not exist for $\delta = 0$, evolution of the stress can be calculated in this limit. For example, in the limit $\delta \to 0$, the stresses in the radial direction are given by

$$\sigma_r^P \sim 4t \ln\left(\frac{R_*}{r_c}\right) - \frac{2\Gamma}{R_*}, \tag{37}$$

$$\sigma_r^Q \sim 4t \ln\left(\frac{R_*}{r}\right) - \frac{6}{\lambda}\ln^2\left(\frac{r}{r_c}\right) - \frac{2\Gamma}{R_*}, \tag{38}$$

$$\sigma_r^C \sim 4t \ln\left(\frac{R_*}{r_c}\right) + \frac{2\lambda t^2}{3} - \frac{2\Gamma}{R_*}. \tag{39}$$

These solutions differ insignificantly form those presented in [8] (the differences are due to the different source functions chosen) and show the stress growing linearly with time.

The preceding solutions hold until $P = 0$, when necrosis forms. Since the pressure decreases monotonically with depth penetrated into the tumour this will first occur at $r = 0$. So, for example, since the illustrative profiles given in figures 2—4 show that the pressure in the tumour interior is negative from an early point it is expected that necrosis will form well before the illustrated equilibrium stress profiles are achieved.

3.2. Critical parameter regimes

If $\delta \neq 0$, then as $t \to \infty$ a steady state stress distribution will be achieved and if necrosis is to form it must do so before this occurs. Thus by looking at the equation $P^C = 0$ in the limit $t \to \infty$ the bounds of the parameter regime in which necrosis can form can be investigated. In the limit $\lambda \to 0$, the steady state outer radius given in (28) can be approximated by

$$R_* = -\frac{3\ln(\alpha)}{\lambda} + \mathcal{O}(1), \tag{40}$$

and the equation $P^C = 0$ by

$$\frac{4}{\delta}\left[\ln\left(\frac{3}{3-\lambda}\right) + \frac{\lambda}{3\delta}\right] + \frac{2\Gamma\lambda}{3\ln(\alpha)} \sim 0, \tag{41}$$

Upon rearrangement of (41) the critical value of $\delta = \delta_{\text{crit}}$ above which necrosis will not form can be found in terms of Γ and α,

$$\delta_{\text{crit}} \sim -\frac{\ln(\alpha)}{\Gamma\lambda}\left[3\ln\left(\frac{3}{3-\lambda}\right) + \sqrt{9\ln^2\left(\frac{3}{3-\lambda}\right) - \frac{2\Gamma\lambda^2}{\ln(\alpha)}}\right]. \tag{42}$$

In the limit $\lambda \to 0$ this gives

$$\delta_{\text{crit}} \sim -\frac{1}{X}\left[1 + \sqrt{1-2X}\right], \tag{43}$$

where $X = \Gamma/\lambda$. Consequently, depending on the relative sizes of Γ and λ, necrosis can form in a Maxwell viscoelastic fluid with any relaxation time, with necrosis forming in a purely viscous material only when the surface tension coefficient Γ is sufficiently small.

3.3. Critical time to necrosis

If necrosis is to form, then it will do so at a critical time, $t = t_{\text{c}}$, and this time can be calculated from the equation $P^C(t = t_{\text{c}}) = 0$. In order to find a leading order approximation to t_{c} we solve the following transcendental equation

$$\frac{\lambda t_c}{3}e^{-\delta t_c} - (1 - e^{-\delta t_c})\left[\ln\left(\frac{3}{3-\lambda}\right) + \frac{\lambda}{3\delta}\right] - \frac{\Gamma\lambda\delta}{6\ln(\alpha)} \sim 0. \tag{44}$$

For example, taking the limit $\delta \to 0$ in (44) gives $t_c = \mathcal{O}(\delta)$, showing that, in a purely elastic spheroid, necrosis forms immediately and the equations of motion should be solved subject to boundary conditions on an inner necrotic interface from the outset. Furthermore we note that, to leading order, t_c is linear in both the surface tension coefficient, Γ, and the death rate, λ, but insensitive to α, the critical nutrient concentration at which cells start to die.

4. Discussion

In this paper we have developed a mathematical model for tumour growth which incorporates growth induced stress generation to examine development of spheroid architecture. In a MCTS with no necrotic core the position of the outer surface of the tumour can be determined by the equation of mass conservation alone and the particular rheology of the tumour is incidental. In the case of growth limited by a single nutrient, the traditional view is that as levels of this nutrient become critically low the cells die and necrosis forms. Within this framework, the specific rheology of the tumour is again incidental since the position of the necrotic interface can also be determined by mass conservation alone. However, observations by Helmlinger et al. [6] and work by Chen et al. [3] has shown that external stress transmitted through the cells and extra-cellular matrix may have a significant effect on development of spheroid architecture. For this reason, in this paper we have adapted the simple model for necrosis formation proposed by Please et al. in [16] which takes into account the specific tumour tissue rheology and have applied it to modelling the formation of a necrotic region in a Maxwell viscoelastic material. In doing so we have shown that a non-uniform cellular proliferation rate, based upon availability of nutrient, and a low death rate in the tumour interior leads not only to spheroid growth, but also to the generation of an evolving, non-uniform, stress distribution. It has previously been shown by Jones et al. [8] that for a tumour consisting of a purely elastic material no steady state stress distribution can exist within a steady state size tumour. Of course such stress evolution is not seen experimentally and this was seen as a weak point of their model. In this paper we have considered a solution to this problem in two ways. Firstly, by the consideration of nutrient dependent growth in a Maxwell viscoelastic spheroid we have shown that the addition of any amount of viscosity to the system will allow for the formation of a steady state stress profile within an equilibrium size tumour, by allowing some stress relaxation, particularly in the azimuthal directions. Secondly, by consideration of a model for necrosis formation based upon force balances within the tumour we have shown that necrosis may form immediately in a tumour consisting of a purely elastic material. Consequently the equations of motion should be solved subject to boundary conditions which ensure necrosis in regions of tension from the outset. This will, of course, limit the tensile stress buildup within the tumour and allow for more physically reasonable results to be obtained.

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