Mathematical model of macrophage-facilitated breast cancer cells invasion

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HIGHLIGHTS

- We model paracrine and autocrine signalling between breast tumor cells and macrophages.
- Analytical results are confirmed with discrete and continuum model simulations.
- Motility of breast tumor cells can be eliminated by changing certain model parameters.
- We identify parameters responsible for the observed tumor cell to macrophage ratio.
- Sensitivity of tumor cells to autocrine signalling affects their ability to migrate.

ARTICLE INFO

Article history:
Received 15 June 2013
Received in revised form 24 February 2014
Accepted 24 April 2014
Available online 6 May 2014

Keywords:
Paracrine signalling
Linear stability analysis
Chemotaxis equation
Metastasis
Discrete model

ABSTRACT

Mortality from breast cancer stems from its tendency to invade into surrounding tissues and organs. Experiments have shown that this metastatic process is facilitated by macrophages in a short-ranged chemical signalling loop. Macrophages secrete epidermal growth factor, EGF, and respond to the colony stimulating factor 1, CSF-1. Tumor cells secrete CSF-1 and respond to EGF. In this way, the cells coordinate aggregation and cooperative migration. Here we investigate this process in a model for in vitro interactions using two distinct but related mathematical approaches. In the first, we analyze and simulate a set of partial differential equations to determine conditions for aggregation. In the second, we use a cell-based discrete 3D simulation to follow the fates and motion of individual cells during aggregation. Linear stability analysis of the PDE model reveals that decreasing the chemical secretion, chemotaxis coefficients or density of cells or increasing the chemical degradation in the model could eliminate the spontaneous aggregation of cells. Simulations with the discrete model show that the ratio between tumor cells and macrophages in aggregates increases when the EGF secretion parameter is increased. The results also show how CSF-1/CSF-1R autocrine signalling in tumor cells affects the ratio between the two cell types. Comparing the continuum results with simulations of a discrete cell-based model, we find good qualitative agreement.

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1. Introduction

In breast cancer, the presence of macrophages at the tumor site is related to poor prognosis (Condeelis and Pollard, 2006; Lewis and Pollard, 2006). This is surprising since macrophages are a part of our immune system. However, studies have shown that macrophages play various roles in tumor development and progression, one of which is to increase tumor cell motility. Tumor cells and macrophages in close proximity communicate via a short-ranged chemical signalling loop based on epidermal growth factor, EGF, secreted by macrophages, and colony stimulating factor 1, CSF-1, secreted by tumor cells (Lin et al., 2001, 2002; Goswami et al., 2005; Condeelis and Pollard, 2006; Lewis and Pollard, 2006; Wyckoff et al., 2007; Beck et al., 2009; Patsialou et al., 2009). This signalling results in aggregation (van Netten et al., 1993), so that tumor cells migrate alongside macrophages toward blood vessels or surrounding tissues and organs (Wyckoff et al., 2007). Extravasation (escape out of a blood vessel) results in metastasis, the formation of secondary tumors, a primary cause of death in breast cancer patients. Hence, limiting or eliminating tumor cell motility is a crucial part of cancer treatments. Experiments have shown that when the number of macrophages is decreased at breast...
cancer sites, tumor progression is slower and fewer cells are able to metastasize, resulting in increased survival rates (Lin et al., 2001). Here we use mathematical modelling to examine interactions between tumor cells and macrophages in a nutrient-rich in vitro situation.

In order for the tumor cell-macrophage interactions to result in group migration, the cells must have a net tendency for aggregation. By aggregation, we mean the tendency of the system to develop a nonuniform spatial distribution where cell clusters form. The density of such clusters is generally well-elevated over background densities, though we do not claim a specific density or size in order to call such a cluster “an aggregate”. Some clues about the underlying process are provided by quantitative measurements. For example, in Wyckoff et al. (2004) it is found that the ratio of tumor cells to macrophages is 3 to 1 in experiments conducted in mice where cells were collected into micro-needles containing EGF. In Patsialou et al. (2009) a similar experiment is conducted using a human breast cancer cell line and the ratio between tumor cells and macrophages increases to 15 to 1. In this paper we explore how features of the paracrine and autocrine signalling loops contribute to this tendency. Principally, we ask the following questions:

- Under what conditions is the paracrine loop sufficient to produce aggregation of tumor cells and macrophages?
- How does the size of an aggregate depend on aspects of signalling such as rates of secretion?
- What signalling aspects are most easily changed by drugs to eliminate aggregation?
- How would treatment by drugs affect the process?
- What are the key differences between various cancer cell lines?
- What governs the observed 3 to 1 ratio between motile tumor cells and macrophages?

To address these questions, we introduce two models for interactions and motility of tumor cells and macrophages. The first, described in Section 2.1, is a continuous 1D Eulerian model, amenable to both analysis and simulations. The second (Section 2.2) is a discrete Lagrangian model where individual cells are tracked. In Section 3 we adapt methods from Luca et al. (2003) and Green et al. (2010) to perform a linear stability analysis of the Eulerian model. This leads to conditions for spontaneous aggregation of cells. Results of full simulations of the PDEs and of the discrete model are presented in Section 5. The advantages of this dual approach are that we can use analytical PDE tools to understand parameter dependence (using the continuum model) while preserving our ability to track individual cells and how they move (using the discrete simulation). We summarize our findings in Section 6 and discuss how our models can be useful for designing cancer treatments.

1.1. Paracrine loop and experimental results

Macrophages are a type of white blood cell comprising approximately 5% of the body's white blood cell count. Macrophages originate from monocytes circulating in the bloodstream and are recruited to tumor sites by chemotactic factors such as the colony stimulating factor-1, CSF-1 (Lewis and Pollard, 2006). Up to 50% of the cell mass in breast tumors can be macrophages (Lin et al., 2002). In Table 1 we provide information on cancer cell lines of interest here.

Tumor cells manipulate innate macrophage signalling in order to migrate. The tumor cells secrete CSF-1, which can bind to CSF-1 receptors on macrophages. This activates the macrophages to chemotax towards a CSF-1 gradient and to secrete EGF. The EGF can then bind to receptors on tumor cells, continuing the chain of activation. Activated tumor cells respond by secreting more CSF-1 and chemotacting in the direction of the EGF gradient (Beck et al., 2009; Pu et al., 2007; Goswami et al., 2005; Wyckoff et al., 2004). This process results in a short-ranged chemotactic signalling loop, also called a paracrine loop, see Fig. 1.

The first indication of a macrophages role in tumor cell motility was provided by van Netten et al. (1993). In their experiment, macrophages and tumor cells plated together form multicellular aggregates within 24 h. Wyckoff et al. (2004) conducted in vivo experiments in mice to study motility and intravasation (crossing into blood vessels) of tumor cells and macrophages. They used PyMT-induced mammary tumors and a multi-photon microscope to view the process. Tumors were grown for 16–18 weeks after which the anaesthetized mice were viewed under a microscope. Collection needles containing 25 nM EGF were placed inside the tumor. In 4 h, approximately 1000 cells were collected, with 73% tumor cells and 26% macrophages. This ratio of approximately 3:1 tumor cells to macrophages was also observed when MTLn3 cells were grown in rats.

Patsialou et al. (2009) showed that, in addition to the paracrine loop, there can also be a CSF-1/CSF-1R autocrine signalling loop (tumor cells both secrete and respond to CSF-1 gradients). This appears to be the case in some human breast cancer cell lines such as MDA-MB-231, which have CSF-1 receptors in addition to EGF receptors. Results from both in vivo (human tumor cells transplanted into mice) and in vitro experiments reported in Patsialou et al. (2009) indicate that invasion of the MDA-MB-231 cell line is less dependent on the macrophages. For example, in micro-needles, only 6% of the collected cells were macrophages (compared to 25% in the experiments with MTLn3 and PyMT).

Motivated by van Netten et al. (1993), we will examine conditions necessary for aggregation of tumor cells and macrophages. In view of Wyckoff et al. (2004), we will also explore what

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Type</th>
<th>Characteristic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyMT</td>
<td>Mouse tumor cell</td>
<td>Highly metastatic (lymph nodes and lungs)</td>
<td>Guy et al. (1992), Wang et al. (2004)</td>
</tr>
<tr>
<td>MTLn3</td>
<td>Rat tumor cell</td>
<td>Highly invasive and metastatic cell line</td>
<td>Henkels et al. (2011)</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Human tumor cell</td>
<td>Largest known number of EGF receptors per cell also has CSF-1 receptors</td>
<td>Henkels et al. (2011), Price et al. (1999)</td>
</tr>
</tbody>
</table>

Fig. 1. Macrophages and tumor cells can interact via a short ranged EGF/CSF-1 signalling loop. Tumor cells and macrophages secrete CSF-1 (respectively EGF) and express EGF (respectively CSF-1) receptors. Each cell type responds to the signal from the other type by moving in the direction of the gradient and by secreting its own signal. This paracrine signalling enables tumor cells to migrate with macrophages away from the primary tumor and towards blood vessels or surrounding tissues.
features of the signalling loop control the ratio of the two cell types observed in such aggregates. We will investigate this in our simulations by introducing a localized source of EGF and observing how this ratio changes as model parameters are varied. Motivated by Patsialou et al. (2009), we will use our model to investigate how autocrine signalling affects the ratio of cell types in the aggregates.

2. Models

Keller and Segel first used partial differential equations to study chemotaxis in 1970 (Keller and Segel, 1971) to investigate aggregation of the slime mold Dictyostelium discoideum. In their analysis, Keller and Segel associated instability in the system with spontaneous aggregation of cells (Keller and Segel, 1970). Since then, similar models have been used to study a wide variety of systems that involve chemotaxis (Sherrat, 1994; Lapidus and Schiller, 1974; Luca et al., 2003; Green et al., 2010). In our first model, we use a set of chemotaxis and reaction–diffusion partial differential equations to account for the motility of breast tumor cells and macrophages and to describe the paracrine signalling loop between the two cell types.

2.1. PDE model

Here we are principally interested in an in vitro culture of cells, where a fixed population of tumor cells interacts with a fixed population of macrophages in a tissue-culture experiment. We consider a 1D spatial domain, \(0 \leq x \leq L\), where \(L\) varies from 4.5 to 45 mm. Let \(\phi(x, t)\) denote the density of macrophages, \(T(x, t)\) the density of tumor cells, \(C(x, t)\) the CSF-1 concentration and \(E(x, t)\) the EGF concentration. We assume that the extracellular matrix is uniform and cell–cell adhesion is neglected. Then the following set of four PDEs captures the EGF/CSF-1 paracrine signalling pathway between the two cell types as well as the CSF-1/CF-1R autocrine signalling in tumor cells:

\[
\frac{\partial \phi}{\partial t} = \mu \frac{\partial^2 \phi}{\partial x^2} - \partial_x \left( \chi_1 \phi \frac{\partial \chi}{\partial x} \right), \tag{1}
\]

\[
\frac{\partial T}{\partial t} = \mu \frac{\partial^2 T}{\partial x^2} - \partial_x \left( \chi_2 T \frac{\partial \phi}{\partial x} \right) - \frac{\partial}{\partial x} \left( \chi_T \frac{\partial C}{\partial x} \right). \tag{2}
\]

\[
\frac{\partial C}{\partial t} = \frac{D}{\partial x^2} \left( \frac{\partial^2 C}{\partial x^2} + s_1 T - \gamma_1 C. \right) \tag{3}
\]

\[
\frac{\partial E}{\partial t} = \frac{D}{\partial x^2} \left( \frac{\partial^2 E}{\partial x^2} + s_2 \phi - \gamma_2 E \right). \tag{4}
\]

The cells move randomly (with random motility coefficient \(\mu\)). Since tumor cells and macrophages move with similar velocities in the experiments, we assume that they have the same random motility coefficient. Cells chemotact towards the signalling chemical gradients (with chemotaxis coefficients \(\chi_1\) for macrophages and \(\chi_2\) for tumor cells). Some cancer cell lines also have a CSF-1/CSF-1R autocrine signalling loop, indicated by the chemotaxis term with coefficient \(\gamma_2\). The two signalling molecules are assumed to diffuse in the domain (with the same diffusion coefficient \(D\)). Macrophages secrete EGF at a rate \(s_2\) and tumor cells secrete CSF-1 at a rate \(s_1\). The two signalling molecules are degraded uniformly at rates \(\gamma_1\) for CSF-1 and \(\gamma_2\) for EGF. Over the timescale of interest (a few hours), cell division and cell death can be neglected. Definitions and default values of all parameters are provided in Table 2.

We impose no flux boundary conditions appropriate to the finite domain in vitro system under consideration. Therefore

\[
\left. \left( \frac{\partial \phi}{\partial x} \right) \right|_{0,L} = 0, \quad \left. \left( \frac{\partial C}{\partial x} \right) \right|_{0,L} = 0, \tag{5}
\]

\[
\left. \left( \frac{\partial T}{\partial x} - \chi_2 T \frac{\partial \phi}{\partial x} \right) \right|_{0,L} = 0, \quad \left. \left( \frac{\partial E}{\partial x} \right) \right|_{0,L} = 0. \tag{6}
\]

This implies that cells neither enter nor leave a domain of size \(L\).

2.1.1. Homogeneous steady state and dimensionless model

Since there is neither birth nor death of cells in the model, the total cell population does not increase nor decrease, but merely redistributes over space. The system can be initialized with various densities of tumor cells and macrophages. For a given average cell density of each type \(\langle n, \phi \rangle\), there is a homogeneous steady state solution (denoted by tildes) where

\[
\tilde{T}_1 = \gamma_1 \tilde{C} \Rightarrow \tilde{C} = \frac{\tilde{T}_1}{\gamma_1}, \tag{7}
\]

\[
\tilde{\phi} = \gamma_2 \tilde{E} \Rightarrow \tilde{E} = \frac{\tilde{\phi}}{\gamma_2}. \tag{8}
\]

Thus, the homogeneous steady state of CSF-1 and EGF depends on the total average density of tumor cells and macrophages, respectively, as well as the chemical secretion and degradation rates. Experiments have shown that up to 50% of the tumor mass can be macrophages. We will therefore assume that half of the cells in our simulations are tumor cells and half are macrophages.

We first consider the default case, where there is no autocrine signalling loop, and where consequently, \(\chi_2 = 0\). We simplify the PDE system and reduce the number of parameters by recasting the equations in a dimensionless form. Denoting the dimensionless variables with * we make the following substitutions in Eqs. (1)–(4):

\[
x = x^* \tilde{x}, \quad t = \tau^* \tilde{t}, \quad \phi = \phi^* \tilde{\phi}, \quad \gamma = \tau^* \tilde{T}, \quad C = C^* \tilde{C}, \quad E = E^* \tilde{E}. \tag{9}
\]

We use the EGF decay time (weighted by the ratio of diffusion rates of chemicals and cells) and the typical distance for EGF diffusion (over its mean decay time) to scale time and distance. The steady state chemical concentrations are used to scale EGF and CSF-1 concentrations. This judicious choice of scaling parameters

<table>
<thead>
<tr>
<th>Parameter set</th>
<th>Symbol</th>
<th>Units</th>
<th>Value</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random motility of cells</td>
<td>(\mu)</td>
<td>([\mu m^2/min])</td>
<td>6.0</td>
<td>Goswami et al. (2005), Kim and Friedman (2010)</td>
</tr>
<tr>
<td>Chemotaxis coefficients</td>
<td>(\chi_1, \chi_2)</td>
<td>([\mu m^2/(min nM)])</td>
<td>1000</td>
<td>Kim and Friedman (2010)</td>
</tr>
<tr>
<td>Diffusion of chemicals</td>
<td>(D)</td>
<td>([\mu m^2/min])</td>
<td>10,000</td>
<td>Kim and Friedman (2010), Thorne et al. (2004)</td>
</tr>
<tr>
<td>Secretion of CSF-1</td>
<td>(s_1)</td>
<td>([nM \mu m^2/(min cells)])</td>
<td>9.6</td>
<td>Estimated</td>
</tr>
<tr>
<td>Secretion of EGF</td>
<td>(s_2)</td>
<td>([nM \mu m^2/(min cells)])</td>
<td>6.9</td>
<td>Estimated</td>
</tr>
<tr>
<td>Degradation of CSF-1</td>
<td>(\gamma_1)</td>
<td>([1/min])</td>
<td>(3 \times 10^{-2})</td>
<td>Morgan et al. (1987)</td>
</tr>
<tr>
<td>Degradation of EGF</td>
<td>(\gamma_2)</td>
<td>([1/min])</td>
<td>(1.5 \times 10^{-2})</td>
<td>Sorkin and Duex (2010)</td>
</tr>
</tbody>
</table>
leads to

\[ \ddot{z} = \frac{D}{\gamma z}, \quad \ddot{t} = \frac{D}{\gamma z}, \quad \ddot{\phi} = \phi, \quad \ddot{T} = T, \quad \ddot{C} = \frac{T_{S1}}{\gamma T}, \quad \ddot{E} = \frac{\phi T_{S2}}{\gamma T} \]  

(10)

With the above choices, the following dimensionless equations are obtained (where the * notation has been dropped for simplicity):

\[ \frac{d\phi}{d\tau} = \frac{\partial^2 \phi}{\partial \xi^2} \frac{d}{\partial \xi} (A_1 \frac{\partial C}{\partial \xi}), \]  

(11)

\[ \frac{dT}{d\tau} = \frac{\partial^2 T}{\partial \xi^2} \frac{d}{\partial \xi} (A_2 \frac{\partial E}{\partial \xi}), \]  

(12)

\[ \frac{dC}{d\tau} = \frac{\partial^2 C}{\partial \xi^2} + \alpha^2 (T - C), \]  

(13)

\[ \frac{dE}{d\tau} = \frac{\partial^2 E}{\partial \xi^2} + (\phi - E). \]  

(14)

The new coefficients are ratios of the original parameters:

\[ A_1 = \frac{\chi \hat{T}_{S1}}{\gamma T}, \quad A_2 = \frac{\chi_2 \hat{T}_{S2}}{\gamma T}, \quad \alpha^2 = \frac{\gamma T}{\gamma T}, \quad \epsilon = \frac{\mu}{D} \]  

(15)

Note that \( \epsilon \) is a very small parameter since the signalling molecules diffuse on a much faster timescale than the random motion of the cells. By writing the equations in this dimensionless form, we reduce the number of parameters from 8 to 4. We can express the new parameters \( A_1 \) and \( A_2 \) as two ratios namely

\[ A_1 = f_1 g_1, \quad A_2 = f_2 \chi_2 \hat{T} / \mu, \quad f_1 = \chi_1 \hat{T} / \mu, \quad g_1 = s_1 / \gamma. \]

The \( f_1 \)'s are ratios of directed and random motility parameters and the \( g_1 \)'s are ratios of production and decay rates of the signalling molecules. Cell lines that are more sensitive to gradients of signalling molecules would have a higher \( f_1 \) value. The \( f_2 \) value would also vary depending on the density of the two cell types. On the other hand, \( g_1 \) might vary between cell lines whose rates of secretion may differ.

2.1.2. Reduction to a system of two equations

Based on the smallness of \( \epsilon \), it is reasonable to consider a quasi-steady state (QSS) approximation for \( C \) and \( E \). This approximation holds since chemicals diffuse on a much faster timescale than the motion of the cells. Eqs. (13) and (14) become

\[ 0 \approx \frac{\partial^2 C}{\partial \xi^2} + \alpha^2 (T - C), \]  

(16)

\[ 0 \approx \frac{\partial^2 E}{\partial \xi^2} + (\phi - E). \]  

(17)

Green’s functions can be used as solutions to these equations (Lee et al., 2001; Luca et al., 2003). Namely,

\[ C = \frac{a}{\alpha} \int_{-\infty}^{\infty} e^{-\alpha x - \gamma T(x)} dx \equiv K_1 \phi T, \]  

(18)

\[ E = \frac{1}{\alpha} \int_{-\infty}^{\infty} e^{-(\gamma - \alpha) x} \phi(x) dx \equiv K_2 \phi. \]  

(19)

These are convolution integrals where \( K_1(x) \) and \( K_2(x) \) represent the exponential kernels \( e^{-\alpha x} \) and \( e^{-(\gamma - \alpha) x} \) respectively. The signalling molecule concentrations are now presented as functions that decrease exponentially with distance away from the cells that secrete them. Concentration of EGF is represented with respect to the distance \( |x - x'| \) to a small group of macrophages of “mass” \( \phi(x') \) and concentration of CSF-1 is represented with respect to the distance \( |x - x'| \) to a small group of tumor cells of “mass” \( T(x') \). Using these Green’s functions, the system can now be reduced to two integro-PDEs containing convolution integral terms:

\[ \frac{d\phi}{d\tau} = \frac{\partial \phi}{\partial \xi^2} - A_1 \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_1 \phi T) \right), \]  

(20)

\[ \frac{dT}{d\tau} = \frac{\partial T}{\partial \xi^2} - A_2 \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_2 \phi) \right). \]  

(21)

Linear stability analysis of this reduced model and comparison to numerical results of the full PDE system are described in Sections 3 and 5 respectively.

2.1.3. Autocrine signalling loop

If we include the CSF-1/CSF-1R autocrine signalling then the dimensionless equation (12) becomes

\[ \frac{dT}{d\tau} = \frac{\partial T}{\partial \xi^2} - \frac{\partial}{\partial \xi} \left( A_2 \frac{\partial C}{\partial \xi} \right) - \frac{\partial}{\partial \xi} \left( A_3 \frac{\partial E}{\partial \xi} \right), \]  

(22)

where \( A_3 = \chi_1 T_{S1} / (\gamma T) \). Using Green’s functions for the concentration of CSF-1 and EGF, Eq. (22) becomes

\[ \frac{dT}{d\tau} = \frac{\partial T}{\partial \xi^2} - A_2 \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_2 \phi) \right) - A_3 \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_1 \phi T) \right). \]  

(23)

Implications of this additional signalling loop are discussed in Section 3.1.

2.2. 3D discrete model

Basic elements of the discrete model are shared with Palsson’s 2007 simulations (Palsson, 2008) for cAMP signalling and cell motility in Dictyostelium discoideum. A summary of the simulation platform is provided in the Appendix. Here we have included two cell types, a paracrine signalling system (Knútsdóttir, 2012) and two signalling molecules, CSF-1 and EGF. The cells are modelled mechanically as individual deformable ellipsoids that exert forces on one another. The concentration of signalling molecules is recorded on a 3D lattice grid and evolves via reaction–diffusion partial differential equations (3) and (4) solved in each lattice grid cube. At each time step in the simulation, forces acting on each cell are summed and the cells moved accordingly on a grid-free 3D domain. The cells move with a constant velocity either in a random direction or, if the signalling molecule gradient across the cell’s diameter is above a set steepness threshold, in the direction of a molecular gradient.

3. Linear stability analysis of continuum model

An advantage of the Eulerian model is that it is amenable to analytic methods. Here, we carry out a linear stability analysis of the simplified model consisting of Eqs. (20) and (21) to explore conditions for spontaneous aggregation. We ask whether a small perturbation \( (\phi^*, T^*) \) from the spatially uniform steady state can lead to aggregation. Consequently we substitute the forms

\[ T = T^* + T_0 e^{\alpha x + \sigma T}, \quad \phi = \phi^* + \phi_0 e^{\alpha x + \sigma T} \]

into (20) and (21) to obtain the linearized system:

\[ \frac{d\phi^*}{d\tau} = \frac{\partial \phi^*}{\partial \xi^2} - A_1 \phi^* \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_1 \phi^* T) \right), \]  

(24)

\[ \frac{dT^*}{d\tau} = \frac{\partial T^*}{\partial \xi^2} - A_2 \phi^* \frac{\partial}{\partial \xi} \left( \frac{\partial}{\partial \xi} (K_2 \phi^*) \right). \]  

(25)

We consider the specific form of perturbations:

\[ T = T^* + T_0 e^{\alpha x + \sigma t}, \quad \phi = \phi^* + \phi_0 e^{\alpha x + \sigma t}. \]  

(26)
Here, \( q \) is the wavenumber, \( \sigma \) is the growth rate of the perturbation and \( T_0 \) and \( \phi_0 \) are the small perturbation amplitudes. This reduces the problem to an algebraic system of linear equations.

We find the Fourier transform of the convolution terms \( K_1 \ast T' \) and \( K_2 \ast \phi' \), (Luca et al., 2003; Green et al., 2010) and calculate the relevant derivatives to obtain the following Jacobian:

\[
J = \begin{bmatrix}
\sigma + q^2 - \frac{\partial^2 A_1 \phi \hat{T}}{q^2 + \sigma} \\
- \frac{\partial^2 A_1 \phi \hat{T}}{q^2 + \sigma} \\
\sigma + q^2
\end{bmatrix}.
\]

(27)

For non-trivial solutions, the determinant of the Jacobian must be zero:

\[
\det(J) = 0 = \sigma^2 + 2q^2\sigma + q^4 \left( 1 - \frac{\partial^2 A_1 A_2 \phi \hat{T}}{(q^2 + \sigma)(q^2 + 1)} \right).
\]

(28)

This equation is of the form \( \sigma^2 + B\sigma + C = 0 \), where

\[
B = 2q^2,
\]

\[
C = q^4 \left( 1 - \frac{\partial^2 A_1 A_2 \phi \hat{T}}{(q^2 + \sigma)(q^2 + 1)} \right).
\]

(29)

(30)

Perturbations grow, which implies that spontaneous aggregation takes place when \( \sigma > 0 \). This requires \( C < 0 \), which leads to the following inequality:

\[
f(q^2) = (q^2 + \sigma^2)(q^2 + 1) < \partial^2 A_1 A_2 \phi \hat{T} \equiv A.
\]

(31)

In the limit \( q \to \infty \), we have \( C > 0 \) so that perturbations with large wavenumbers will not lead to instability.

The inequality (31) specifies the parameter regime where instability and thus spontaneous aggregation of cells can occur in response to small perturbations of the uniform steady state (Fig. 2). It also predicts the range of unstable wavenumbers for a given set of parameters. Instability will first occur for long wavelengths \( q=0 \) provided that \( 1 < A_1 A_2 \phi \hat{T} \). This inequality is satisfied if \( A_1 \) and/or \( A_2 \) are sufficiently large or if cell densities are sufficiently high.

Recall that \( A_i = \chi S_i / \gamma_i \). Thus, inhibiting one or another of the secretion or chemotaxis rates will block aggregation. This is intuitively clear, since such inhibition removes or depresses a part of the signalling loop between the cells. Aggregation is also prevented if one of the chemical degradation coefficients is too large. High degradation coefficient reduces the concentration of the given signalling molecule, limiting the range over which the cells can communicate. At sufficiently low cell density, in early stages of cancer or before macrophages are recruited, no aggregation would be observed.

### 3.1. Autocrine signalling

In the case where the autocrine signalling loop is present, as in (23) with \( A_3 \neq 0 \), LSA leads to the modified Jacobian:

\[
J = \begin{bmatrix}
\sigma + q^2 - \frac{\partial^2 A_1 \phi \hat{T}}{q^2 + \sigma} \\
- \frac{\partial^2 A_1 \phi \hat{T}}{q^2 + \sigma} \\
\sigma + q^2
\end{bmatrix}.
\]

(32)

The condition \( C < 0 \) now leads to the modified instability condition:

\[
(q^2 + \sigma^2)(q^2 + 1) - \partial^2 A_1 A_2 \phi \hat{T} + \partial^2 A_3 \phi \hat{T}.
\]

(33)

It is evident from (33) that including the autocrine signalling loop increases the likelihood of instability in the system and promotes aggregation of cells for all wavenumbers, \( q \). If \( A_3 = 0 \) this equation reduces to the previous inequality, (31), as expected.

### 4. Parameter estimates

We used experimental literature, where available, to quantify parameters (Table 2). However, as such data is sparse, we also rely on previous estimates of some rates and values from other modelling papers, e.g. Kim and Friedman (2010), a common custom in the field of cancer modelling. We use a default cell density of \( 10^4 \) cells/cm\(^2\), based on the density used in experiments (Kim and Friedman, 2010; Liu et al., 2009). The rates of motility associated with the two cell types, \( \mu_1 \) and \( \chi_0 \), are estimated from parameters associated with transformed epithelial cells in Kim and Friedman (2010). Since tumor cells and macrophages are observed to move alongside one another, we assume these parameters to be the same for both cell types. The diffusion coefficient of EGF was measured in the extracellular space of a rat brain by Thorne et al. (2004). The molecular weights of CSF-1 and EGF are \( M_1 = 60.1 \) and \( M_2 = 6.6 \) kDa respectively. The diffusion rates of the two signalling molecules would scale roughly by a factor of \( (M_1/M_2)^{1/2} \approx (60.1/6.6)^{1/2} \approx 2 \), meaning that they are the same order of magnitude and, for simplicity, we assume that they are equal in our model. The degradation coefficients are estimated from experiments, taking into account endocytosis and ligand half-life. The secretion coefficients are calculated using Eqs. (7) and (8), where the average concentrations of CSF-1 (\( \tilde{C} = 0.16 \) nM Scholl et al., 1996) and EGF (\( \tilde{E} = 0.23 \) nM Wyckoff et al., 2004; Boccardo et al., 2003) in tumors are obtained from the literature. All these model parameters can vary by orders of magnitude in the literature. The parameters in Table 2, used in our simulations, initiate cellular aggregation on a timescale of few hours. The dimensionless parameters used in the simulations are listed in Table 3.

We describe the default behaviour of the model and then consider several variations. First, parameters differ between cell

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_1 )</td>
<td>26.7</td>
</tr>
<tr>
<td>( A_2 )</td>
<td>383</td>
</tr>
<tr>
<td>( q^2 )</td>
<td>2</td>
</tr>
<tr>
<td>( c )</td>
<td>( 6 \times 10^{-4} )</td>
</tr>
</tbody>
</table>
lines and external cues. Second, drugs can also alter the values of some parameters. We report the effect of such parameter variations on the aggregation of cells.

5. Numerical simulations

5.1. Simulations of the continuum model

We solve the system of four dimensionless PDEs, (1)-(4), numerically (using the pdepe solver in MATLAB). We use no-flux boundary conditions, as previously noted. Recall that a given uniform initial tumor cell and macrophage distribution will give rise to a steady state uniform concentration of EGF and CSF-1. Here we start with an initial population of cells and let the chemical concentration evolve via secretion/decay. To induce instability, we impose a small perturbation of the uniform cell distribution by using \( T(x,0) = 1 + 0.01 \cdot x_{\text{rand}} \) and \( \phi(x,0) = 1 + 0.01 \cdot x_{\text{rand}} \), where \(-1 < x_{\text{rand}} < 1\) is a random number. The length of the simulation domain is approximately 8 mm and we use \( \Delta t = 1.0 \) min and \( \Delta x = 2.0 \) μm.

Long-term results, shown in Fig. 3, indicate the possibility for aggregation of clusters in several stages. Here the ratio \( \alpha \) in (31) is large enough that the uniform state is unstable and the two cell types aggregate. Once aggregation is initiated, sharp peaks of cells rapidly form. At this stage, since the solution rapidly diverges from its homogeneous steady state, linear stability analysis no longer predicts interpeak spacing. Once peaks form, they are highly localized, and approximate delta-like peaks. Then, their dynamics lead to merging on a slower timescale, as shown in Fig. 3. The net attraction between delta-like peaks of cell clusters is then well-approximated by the nonlocal kernels, \( K_1(x) \) and \( K_2(x) \). Since these are exponentials of the forms \( e^{-|x|/\alpha} \) and \( e^{-|x|} \), cell clusters that are within a distance of a few multiples of \( 1/\alpha \) from one another will experience an “effective attraction”. (The attractive force between clusters falls off by powers of \( 1/\alpha \), i.e. factors of 0.37, 0.135, and 0.05 at mutual distances of 1, 2, and 3 times \( 1/\alpha \) respectively.)

Seven aggregates have formed within 4 days (around \( t = 0.05 \) in Fig. 3). With time, some of the aggregates merge because of the spatial scale over which the chemical signal travels. For simulations with different \( K_{\text{rand}} \) in the initial conditions, the aggregates will form at different positions. For a smaller \( \alpha \) value, the small random perturbations die out and the system equilibrates at a spatially uniform distribution of all cells and chemicals (results not shown). The results of the simulations are in agreement with predictions of the linear stability analysis previously described. However, it is likely that in actual experiments (in vitro or in vivo) only initial steps in this aggregation process would occur before the model fails to be predictive.

5.1.1. Ratio of cells inside aggregates

Motivated by observations of the 3:1 tumor cells per macrophage ratio in the micro-needle experiments of Wyckoff et al. (2004) we asked how parameters in the model (Eqs. (11)-(14)) influence this ratio. Consequently, we conducted simulations with an externally supplied source of EGF in the centre of a (smaller) 800 μm domain, \( E(1/2, t) = 100 \), to represent the externally supplied 25 nM EGF in the Wyckoff experiments. The EGF concentration is kept fixed over a 2 μm range and, as a result, a gradient of EGF forms as it diffuses outwards. We quantified the number of tumor cells and macrophages in the interior of clusters that evolve, as well as their ratio over a range of the dimensionless parameters. Results are shown in Figs. 4-7. In each case, we use the default value of the dimensionless parameter as a reference value, and show how fold changes in that value affect the cells and their ratio in the aggregates that form at steady state. As detailed below, we find that the cell ratio is sensitive to changes in the chemotactic coefficients and the secretion coefficients.

Fig. 4 demonstrates the effect of \( A_1 \) and \( A_2 \) on aggregate cell densities and cell ratio. The \( \alpha^2 \) parameter is kept constant in these simulations and thus we assume that \( \gamma_1 \) and \( \gamma_2 \) are not changing. In Fig. 4A we see that increasing \( A_1 \) tends to increase macrophage density more sharply than tumor cell density, so that their ratio (dotted curve) decreases. Conversely, decreasing CSF-1 secretion rate (\( s_1 \)) corresponds to a lower \( A_1 \) which in turn corresponds to fewer total cells and a larger ratio according to the figure. This result is in agreement with experiments on a cell line deficient in CSF-1 conducted by Wyckoff et al. (2004), who found fewer total cells collected in micro-needles containing EGF.

In Fig. 4B we find that increasing \( A_2 \) in the simulations results in greater density of tumor cells and macrophages in the

![Fig. 3. Simulations of the continuum model, Eqs. (11)-(14), starting close to a uniform distribution (with superimposed 1% random noise on tumor cell and macrophage densities). All panels are kymographs with time (vertical axis) and position (horizontal axis). Top row: cell densities and bottom row: signal concentrations. See Table 3 for parameter values. The simulation time is over 20 days and the total number of cells of each type is conserved in these simulations.](image-url)
versely, decreasing as in Table 3 (note that rates on the cell densities and their ratio in aggregates. As shown containing EGF decreased to background levels. These experiments, the collection of cells into micro-needles would be similar to lowering $\chi$ (2004) using an EGF receptor inhibitor (PD153035). This inhibition qualitative agreement with experimental results of Wyckoff et al. cell densities in the aggregate. These simulation results are in constant. Parameters as in Table 3.

Fig. 6. As in previous figures, but showing the effect of the percentage of macrophages in the initial cell population. Tumor cell density attains a maximum at around 40% macrophages while the ratio of cell types in aggregates stays constant. Parameters as in Table 3.

aggregate, with a sharper increase in tumor cells. Consequently, the ratio of tumor cells to macrophages increases with $A_2$. Conversely, decreasing $\gamma_2$, which decreases $A_2$, would tend to decrease cell densities in the aggregate. These simulation results are in qualitative agreement with experimental results of Wyckoff et al. (2004) using an EGF receptor inhibitor (PD153035). This inhibition would be similar to lowering $\gamma_2$ and therefore decreases $A_2$. In these experiments, the collection of cells into micro-needles containing EGF decreased to background levels.

We next considered the effect of the signalling chemical decay rates on the cell densities and their ratio in aggregates. As shown in Fig. 5, decreasing the degradation of EGF (which corresponds to increasing both the ratio $\gamma_1/\gamma_2$ and $A_2$) increases the number of cells of both types, but scarcely affects their ratio. The same qualitative results are obtained when the CSF-1 degradation coefficient is decreased (results not shown). Interestingly, according to our simulations, the ratio between the two cell types is not sensitive to changes in the CSF-1 and EGF degradation coefficients.

Finally, we considered how the relative proportions of cells of the two types in the initial conditions would affect their densities and relative ratio in aggregates that form later on. To explore this, we set a fixed total cell density and varied the percentage of macrophages in the mix. Results are shown in Fig. 6. Interestingly, we find a maximal density of tumor cells within aggregates when the proportion of macrophages is 40%. This suggests that a seeding ratio of 1.5:1 between tumor cells and macrophages results in a maximum density of tumor cells in aggregates. This maximum occurs where there is a balance between the number of tumor cells in the simulation and the enhancing motility of the paracrine signalling loop through macrophages. At 40% macrophage density the paracrine loop motility effect is at a maximum. Above 40% there are fewer tumor cells that can follow the signal and below 40% there are fewer macrophages that enhance the motility of the tumor cells. These model predictions are important in view of the fact that the relative density of macrophages at a tumor site varies across patients and across tumors. Furthermore, there are drug treatments, such as a legumain-based DNA vaccine, which are known to decrease the density of macrophages at tumor sites (Luo et al., 2006). Targeting the tumor associated macrophages in experiments with mice resulted in a 60% decrease in lung
metastasis (Luo et al., 2006). In our model, decreasing the density of macrophages from 40% to 15% results in a 63% decrease in tumor cell density in aggregates.

As a final test, we asked how the inclusion of the CSF-1/CSF-1R autocrine signalling term in (22) would affect the cell densities and ratio in aggregates. Results, in Fig. 7, indicate that this modification strongly increases the density of tumor cells in aggregates while hardly affecting macrophages at all. Consequently, including the autocrine term sharply increases the cell ratio. As seen in Fig. 7, the cell ratio increases nearly linearly with increasing value of $A_3$. In experiments reported in Patsialou et al. (2009), cancer cells, with autocrine signalling, were collected at a ratio of 15:1 in contrast to the 3:1 ratio when autocrine signalling is absent. Fig. 8 shows line graphs both with and without the autocrine signalling loop. According to our simulations, a ratio of 15:1 is obtained when $A_3 = 650$. One way to obtain such a large $A_3$ value is to have $\chi_3 = 24 \chi_1$. This suggests that the tumor cells are more sensitive (high $\chi_3$ value) to the CSF-1 gradient than to the EGF gradient. Biologically that can be due to different numbers or distinct activation rates of the two receptor types. The $A_3$ parameter can also be increased by either increasing the secretion of CSF-1, $s_1$, or decreasing the degradation of CSF-1, $\gamma_1$. However, increasing $s_1$ or decreasing $\gamma_1$ would increase the $A_1$ parameter also, which does not increase the ratio between the densities of the two cell types (data not shown).

5.1.2. Repulsion between cells

Chemotaxis models such as the one discussed here are notorious for sharp peaks (Fig. 8B) and, in some cases, solutions that blow up. In a discrete cell-based simulation, this is prevented by a short-ranged repulsion term that prevents cells from overcrowding. We considered the effect of adding a similar local repulsion term to our continuum model:

$$\frac{\partial \phi}{\partial t} = \frac{\partial^2 \phi}{\partial x^2} - A_1 \frac{\partial}{\partial x} \left( \phi \frac{\partial C}{\partial x} + B_1 \frac{\partial (T + \phi)}{\partial x} \right),$$

(34)

$$\frac{\partial T}{\partial t} = \frac{\partial^2 T}{\partial x^2} - A_2 \frac{\partial}{\partial x} \left( T \frac{\partial E}{\partial x} + B_2 \frac{\partial (T + \phi)}{\partial x} \right),$$

(35)

where $B_1$ and $B_2$ are the dimensionless repulsion coefficients. As in Green et al. (2010) we assume that the strength of the repulsion between different cell types is the same ($B_1 = B_2 = 1$). Results, shown in Fig. 9, are comparable to those of Green et al. (2010), where peaks are broader. For $B_1 = B_2 = 1.0$, cells of one type (macrophages) are partly segregated to the exterior of a cluster of the other cell type (tumor cells). When $B_1 = 0.2$ and $B_2 = 2$, for example, segregation does not take place. Similar results were noted in Green et al. (2010).

5.2. Discrete model simulations

The discrete model, described in Section 2.2, is initiated with 1600 cells (in a 1:1 ratio) randomly distributed on a 2D square. The cells can move in the full 3D cube, which we consider to contain extracellular matrix (ECM), where the cells can gain traction, apply active motive forces, and migrate. We do not describe details of cell-ECM interactions in this model. The default diffusion coefficient is $10,000 \mu m^2/min$, the max secretion of EGF and CSF-1 is set to $10^{-3} nM/(m/min \cdot cell)$ and the degradation coefficient is $5 \times 10^{-4} m^{-1}$. In the discrete model, we assume that cells only sense a chemical gradient when it exceeds some threshold (1% across the cell diameter). The gradient threshold replaces the chemotaxis coefficients, $\chi_1$ and $\chi_2$, used in the continuum model. If the chemical gradient is above the set threshold, the cell will orient and chemotax up the gradient at constant velocity $v_{chemotaxis} = 2.8 \mu m/min$. Otherwise, the cell moves in a random direction with constant velocity $v_{random} = 2.0 \mu m/min$. In this respect, the discrete and continuum models differ. Furthermore, in the discrete model, cells cannot occupy the same location, and physical forces between adjoining cells (short-ranged repulsion) prevent such overlap.

Fig. 10 shows two simulation time sequences in which the gradient thresholds take on distinct values. In a region of parameter space consistent with instability (identified by LSA for the continuum model), aggregation occurs (top panels, supplementary video 1), whereas when the gradient threshold is increased to 8% (bottom panels), the cells fail to aggregate. These results are in good agreement with the LSA predictions of the continuum model. In contrast, the 3D discrete model can provide details at the level of single cells. Some sample trajectories of cells moving towards the aggregates are illustrated in Fig. 11.

Decreasing the sensitivity of macrophages to a gradient of CSF-1 (increasing the CSF-1 gradient threshold from 1% to 5%), Fig. 12, results in some cells being left outside of aggregates (Supplementary Video 2). Then there are fewer macrophages in the aggregate and consequently the cell ratio increases. The simulation is over 80 h which is the timeframe of interest in experiments. At longer timescales other cellular behaviours that have not been included in our model, such as cell division and apoptosis, start becoming important in the biological system. Increasing the CSF-1 gradient threshold is similar to decreasing the $A_1$ parameter in the continuum model. The increasing tumor cell to macrophage ratio is in good agreement with results of the continuum model in Fig. 4A.

As a last example, we used the discrete model to simulate the response of cells to an externally supplied signal gradient. Accordingly, we impose an external EGF source in the centre of the

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**Fig. 8.** Vertical axes represent (left) density of tumor cells and macrophages and (right) concentration of EGF and CSF-1. (A) Parameters as in Table 3. There is a ratio of 3 to 1 between the peak cell densities of tumor cells and macrophages. (B) As in (A) but with autocrine signalling and $A_3 = 650$. The ratio increases to 15 to 1. The tumor cell density peak becomes significantly narrower when the autocrine signalling is turned on. $l = 4.5$ mm.
domain which represents the needle in the in vivo experiments (Wyckoff et al., 2004). The EGF concentration is kept fixed at 0.01 nM over a 30 × 30 μm region and allowed to diffuse to form a gradient. 400 cells are initially randomly distributed in a 1:1 ratio in the simulations. As before, we now include a CSF-1/CSF-1R autocrine signalling for the tumor cells. Results in Fig. 13 indicate the needle location as a cylinder into which cells can migrate and crawl up the gradient. In the top figure, case T1 (Supplementary Video 3), the tumor cells are more sensitive to gradients of CSF-1 than in the bottom figure, case T2 (Supplementary Video 4). We find a tumor cell to macrophage ratio close to 1:1 inside the simulated needle in the bottom figure whereas the top figure, with greater gradient sensitivity, results in a ratio closer to 2:1.

6. Discussion

Cancer is a heterogeneous disease where an interplay of multiple chemical and mechanical signals in different cell types takes place. Microscopic events occurring within a cell and mesoscopic events involving cell–cell interactions need to be considered when studying the disease. These events take place at varying time and spatial scales, motivating the need for multi-scale modelling.

Mathematical modelling has been used in cancer research for decades, and has been on the rise in recent years. Many researchers have been concerned with the growth and proliferation of solid tumors, with and without vasculature, as well as metastasis. Drasdo and Hohme (2005) studied a growing tumor using a 2D and a 3D cell-based mechanical simulation, with a focus on how cell division and nutrient limitation in the core affects the overall growth rate. Their discrete simulation method is parallel to our discrete 3D model. A review of cell-based multi-scale models appears in the book Anderson et al., 2007. A comparison of the discrete model (Drasdo and Hohme, 2005) and its analogous continuum model was described in Byrne and Drasdo (2009). A comprehensive review of cancer cell modelling that compares continuous, discrete and hybrid approaches to cancer modelling appears in Chakrabarti et al. (2012). The authors focus on multi-scale models where various sizes and time scales are considered, and discuss advantages and shortcomings of various approaches. Further 3D extension of tumor growth that includes cell division, adhesion, angiogenesis as well as ECM degradation has been modelled in Macklin et al. (2009) and Cristini et al. (2003) using boundary-integral simulations in 3D. None of these studies have considered interactions of tumor cells and macrophages.

Other modelling studies have been concerned with the development of tumor morphology. Formation of 3D multicellular

![Fig. 9.](image9.png) The effect of a repulsion term in Eqs. (34) on densities of (A) macrophages and (B) tumor cells. Macrophages are segregated to the outside of a cluster of tumor cells. \( B_1 = B_2 = 1.0 \), with other parameters as in Table 3. Dotted line (\( t = 0.05 \)): 16 h, dashed line (\( t = 0.1 \)): 32 h and solid line (\( t = 0.15 \)): 48 h.

![Fig. 10.](image10.png) Simulations of the discrete Lagrangian model. Cells are initiated on the 2D surface, but can move in 3D. Top panel: aggregation occurs (gradient threshold is 1%). Bottom panels: no aggregation (gradient threshold is 8%). Tumor cells are in green and macrophages in red. Brighter colour cells are moving towards a chemical gradient. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)
tissue morphologies (normal and cancerous) was simulated using bio-mechanical model of epithelial morphogenesis by Rejniak and Dillon (2007), Rejniak et al. (2010). Both proliferation and cell adhesion were included, using a method based on immersed-boundary computation of cell structure and interaction. In recent years, many revisions to the Keller–Segel chemotaxis equations on which our model is based have been proposed. In some cases, the modified models specifically address the issue of cell crowding, preventing the formation of sharp localized peaks (Murray et al., 2009; Simpson et al., 2011; Markham et al., 2013). Here we considered a repulsion term in place of this alternative. Both models and therapeutic approaches have been based on the idea that macrophages seek out the hypoxic regions associated with a tumor. For example, solid tumor, normal tissue, and macrophages...
killing of aberrant cells were included in PDE models to study spatial heterogeneities that can evolve in Owen and Sherratt (1997, 1998). Similarly, macrophages aggregating towards tumor hypoxia were modelled as a PDE free-boundary problem for the expanding spheroid (cells of both types and extracellular fluid) in Webb et al. (2007). Typically, such models depict both random motion of cells and chemotaxis of macrophages, with parameters for motility of macrophages based on experimental data. A growing avascular tumour spheroid infiltrated by macrophages that lead to increased tumor size was described in Owen et al. (2004). The authors discuss the notion that macrophages could be used as drug-delivery vehicles in therapeutic strategies. This idea is explored more fully in the 2D agent-based detailed models of Owen et al. (2011). These models include cell division, p53-VEGF signalling, development of blood vessels, oxygen and drug levels and application of macrophages in chemotherapy. While all these papers address interactions between tumor cells and macrophages, none of them consider the autocrine and paracrine signalling loops and tandem motion of cells that we discuss here.

For cells in a tumor to aggregate or invade into surrounding tissues, they first need to disassociate from their neighbours. In our models, we assume that the cells have already undergone that process. In a paper by Ramis-Conde et al. (2008a) a multi-scale approach was used to study loss of tumor cell adhesion to other cells. The focus in the paper was on E-cadherin and β-catenin, and how the up- and the down-regulation of these molecules contributed to cell adhesion and hence motility. Another paper by Andasari et al. (2012) introduced a complementary lattice-free approach to Ramis-Conde et al. (2008a), which is comparable to our discrete model. Andasari et al. showed that the results from the two modelling approaches were in good agreement.

In this paper, we used complementary approaches to study the motility of breast cancer cells in the presence of macrophages. The PDE formulation and the continuum version are useful for general conclusions about parameter ranges in which aggregation of cells and formation of clustering can occur. Such PDE formulation allows us to understand parameter dependence and overall global behaviour. Simply recasting the PDEs in a non-dimensional form is informative, showing that ratios of parameter groupings rather than each individual parameter are important. For example, we showed that the model behaviour depends on four effective dimensionless ratios, \( A_1, A_2, a^2 \) and \( c \), (see (15)), rather than values of eight individual parameters. Furthermore, linear stability analysis of the Eulerian version produced the condition for aggregation (inequality (31)), which then gives insight into parameter dependence.

Lagrangian cell-based simulations are computationally intensive, so that multidimensional parameter sweeps are more costly in computation time. That said, complementing the continuum model with cell-based Lagrangian computations allows us to see the trajectories and behaviours of individual cells, and to study interactions in small as well as large cell groups. As in the work of Andasari et al. (2012), we complemented the PDE model with a cell-based approach to compare the mean-field PDE and detailed individual-cell behaviors.

Some of our model result can help in drug design. According to our model predictions, aggregation of tumor cells with macrophages, and consequently group migration and metastasis could be reduced or eliminated by one or more of the following treatments:

- decreasing the secretion of CSF-1 or EGF,
- increasing the degradation of CSF-1 or EGF,
- decreasing the density of macrophages,
- decreasing the chemotaxis sensitivity of tumor cells or macrophages.

Drugs that block one or another of these processes, singly or in combination, would then inhibit the paracrine signalling and aggregation of the tumor cell-macrophage mixture.

The relative proportions of tumor cells and macrophages in aggregates have been noted in a variety of experiments. Here we explored this ratio and how it depends on system parameters, and on the initial proportions of cells. We found that decreasing the parameters \( s_2 \) and \( \chi_2 \) or increasing \( s_1 \) and \( \chi_1 \) led to a reduction in the ratio, whereas changing \( \gamma_1 \) and \( \gamma_2 \) had little effect. Wyckoff et al. (2004) conducted experiments with two different cell-lines, MTLn3 and PyMT, and found that the ratio between tumor cells and macrophages collected in micro-needles was the same, 3 to 1. Our model suggests that the secretion and chemotaxis coefficients for these two cell lines are similar. However, according to our model the degradation of EGF and CSF-1 can be different. Experiments with drugs that decrease the density of macrophages at tumor sites showed a 60% decrease in the number of lung metastasis (Luo et al., 2006). In our simulations, the density of
tumor cells in aggregates decreased when the density of macrophages was decreased. Our simulation results are thus in qualitative agreement with the experimental results.

Addition of the CSF-1/CSF-1R autocrine signalling loop increased the density of tumor cells in the aggregate and hence also increased the ratio of tumor cells to macrophages in the aggregate. These results were in good agreement with results of Patsialou et al. (2009). Comparing the numerical results to the experimental results suggests that in order to obtain the same ratio between tumor cells and macrophages as in the experiments the dimensionless autocrine chemotaxis coefficient needs to be $A_3 = 650$. This indicates that the ratio $A_3/A_1 = x_3/x_1 = 24$, which suggests that the tumor cells are significantly more sensitive to gradients of CSF-1 than to gradients of EGF. Due to the many simplifications in this model, this quantity might be overestimated. It does however suggest that there is a difference in the cells’ sensitivity to EGF and CSF-1 gradients.

Our model and simulations could be validated against specific experimental tests that would extend findings of Wyckoff et al. (2004), for example. We suggest varying the doses of receptor blockers or densities of macrophages. According to our simulations, changing the dose of a drug such as IRESSA should decrease the density of both cell types in aggregates and decrease the tumor cell to macrophage ratio. (IRESSA is an EGFR receptor blocker. Its effect is equivalent to decreasing $A_2$ in our model; see Fig. 4B). Similarly, a CSF-1 receptor-blocking antibody, such as AF598, corresponds to decreasing $A_1$ in our model. Hence, we predict that an experiment with increased doses of AF598 would decrease the density of both cell types in aggregates but increase the ratio of tumor cells to macrophages. According to our simulation results, there is a maximum density of tumor cells in aggregates when the tumor cell to macrophage ratio is 1:5:1. Conducting experiments with different seeding ratios between tumor cells and macrophages in tissue culture could give insight into parameters in the system such as the chemotaxis parameters.

In interpreting and comparing predictions to experimental results, one should also recognize that the model is simplified and has clear limitations. We have not considered heterogeneity of tumor cell behaviour, and the interactions with surrounding normal tissue. Some future improvements of such models may include (a) making the secretion terms sigmoidal and dependent on the concentration or the receptor binding of the other signalling molecule, (b) including membrane-bound degradation terms, (c) including adhesion between cells (see, e.g. Ramis-Conde et al., 2009), (d) including interactions of cells with the inhomogeneous extracellular matrix and/or collagen fibres (see Ramis-Conde et al., 2008b) and (e) including other signalling molecules, such as TGFβ, which can also affect the motility of tumor cells. In principle, these models can also be used to study other systems that depend on paracrine and/or autocrine signalling, such as the motility of glioma cells.

Acknowledgements

The research was supported by an NSERC Discovery Grants to L. E.K. and E.P. The continuum model and its analysis was initiated at an International Graduate Training Center in Mathematical Biology summer graduate course, sponsored by the Pacific Institute for the Mathematical Sciences. This research was enabled in part by support provided by WestGrid (www.westgrid.ca) and Compute Canada Calcul Canad (www.computecanada.ca).

Appendix A. Details of the 3D simulation model

Here, we briefly summarize the 3D simulation platform. Further details can be found in Palsson (2008) and Knutsdottir (2012).

A.1. The chemical concentrations and diffusion equations

While cell positions are continuous, the concentrations of chemicals are represented on a regular 3-D grid (with $\Delta x = \Delta y = \Delta z = 10 \mu m = cell diameter$). The local external concentration at cell $p$, $[C]_{cell,p}$, is the weighted average of values of $[C]_{ijk}$ inside grid cubes $ijk$ overlapping the cell. The interpolation between off-lattice cell nuclei and on-lattice chemical mesh is processed by weighting each grid cube in proportion to the fraction of the cell surface area inside that grid cube:

$$[C]_{cell,p} = \sum_{i,j,k} \sum_{m=1}^{n+1} \sum_{m=1}^{n+1} A_{cell,p} [C]_{ijk}.$$  \hspace{1cm} (36)

$A_{cell,p}^{ijk}$ is the surface area of cell $p$ inside grid cube $ijk$ which is total surface area of cell $p$. Indices $l,m,n$ correspond to the grid cube that contains the $x,y,z$ position of cell $p$ respectively (see Palsson, 2008). Since $l,m,n = 1..3$, the cell can sense a total of 27 grid cubes in determining the local gradient. The diffusion equations for chemical concentrations are solved using an explicit time-stepping iteration that is second-order (in space).

A.2. Cell properties

Cells are represented as viscoelastic deformable ellipsoids. We track the coordinate of the center of mass, the orientation and the principal ellipsoidal axes, $a$, $b$ and $c$ (with lengths $a$, $b$ and $c$). Each axis is depicted by a Kelvin element (nonlinear spring in parallel with a spring and dashpot in series; spring constants are $k_1$ and $k_2$, and $\mu_1$ is the viscosity of the dashpot) (Fung, 1993). The cell volume is conserved, so that compression of one axis results in expansion of another. This generates a counteracting force, $F_{mod}$. The stress–strain relationship for the Kelvin model (Fung, 1993) including $F_{mod}$ leads to the following equations governing deformation of the axis $i$ of a cell under a given force on that axis ($F_i$):

$$\frac{dr_i}{dt} = \frac{k_1(F_i - F_{mod})}{\mu_1(k_1 + K_2)} + \frac{dF_i}{dt} + \frac{r_i}{\mu_1(k_1 + K_2)}$$  \hspace{1cm} (37)

$$r_a r_b r_c = (r_a + \Delta r_a)(r_b + \Delta r_b)(r_c + \Delta r_c) = V_{ellipsoid} \frac{4}{3} \pi r_c^3$$  \hspace{1cm} (38)

where $r_i$ is the length of the given axis (units of 10 $\mu$m). The force $F_{mod}$ is computed every time step based on the volume constraint (by solving (36) to find $\Delta r_a$, $\Delta r_b$ and $\Delta r_c$ under the constraint (37)). This amounts to solving a 3rd order polynomial for $F_{mod}$.

A.3. Forces

In the 3D simulation, cells deform and exert forces on one another, as well as responding to mechanical and chemical signals. Cell resistance to deformation, active cell locomotion, as well as drag all lead to external forces. In cases where a cell applies force to its neighbour, that neighbour experiences an equal and opposite force. Unlike the simulations in Palsson (2008), where adhesion between cells played an important role, here neither tumor cells nor macrophages adhere to one another, and forces are almost exclusively generated by adhesion to the extracellular matrix. The forces acting on a cell consist of active forces, $F_{active}$, and repulsive forces from all the $N$ neighbours of the cell, $F_{repulsive}$:

$$F_{cell} = F_{active} + \sum_{i=1}^{N} F_{repulsive}.$$  \hspace{1cm} (39)

Below we describe the active and repulsive forces acting on cells.
A.3.1. Active force

An active force is generated when a cell starts to move randomly or in response to a chemotactic signal. The cell applies this force to the extracellular matrix. The major axis $a$ of the moving cell is then oriented in the direction of the active force. Force governing random motion is set to be $F_{\text{active}} = 70\%$ of the chemotactic force. Typically, the active chemotactic force is set at $F_{\text{chemotax}} = 10^{-5}$ dyne (1 dyne $= 10^{-5}$ N): \[
F_{\text{active}} = \begin{cases} 
F_{\text{random}} & \text{if } \nabla [E] < \text{threshold level}, \\
F_{\text{chemotax}} & \text{if } \nabla [E] \geq \text{threshold level}.
\end{cases}
\] (40)

A.3.2. Repulsive force

The repulsive forces arise when a neighbouring cell gets too close. We use the distance, $d$, between the surfaces of two ellipsoidal cells to estimate the strength of the repulsive force: \[
F_{\text{repulsive}} = \begin{cases} 
0 & \text{if } x > 0, \\
F_{\text{compress}}(x) & \text{if } x \leq 0.
\end{cases}
\] (41)

Here $F_{\text{compress}}$ is the strength of the repulsive force, $x = d/r_{\text{cell}}$, where $r_{\text{cell}}$ is the radius of the cells (5 μm) and $\mathbf{r}_{ij}$ is a vector between the centre of the two cells.

A.3.3. Equations of motion

At the size-scale of cells, inertial forces are negligible. The active and repulsive forces are thus balanced by the drag force, resulting in the following equation of motion: \[
dx_i/dt = v_i, \quad i = 1, \ldots, N.
\] (42)

Here $\mathbf{v}_i$ is the velocity of cell $i$ and $\mu_{\text{ecm}}$ is the viscosity between the cells and the extracellular matrix.

The system is solved numerically using a time stepping scheme as follows:

Step 1. Calculate the chemical concentrations.

Step 2. For each cell $i$,

(i) Find the direction of the local relevant chemical gradient. If that gradient is above threshold, orient cell $i$ towards the gradient.

(ii) Find all neighbours of cell $i$ (within a minimum distance).

(iii) Calculate and sum active and repulsive forces on cell $i$ due to neighbours. Deform the ellipsoidal axes of cell $i$ (while conserving volume) and move it based on Eq. (42).

Step 3. Randomize the order of cells to be updated in the next time step (avoids spurious results in cellular automata models).

Appendix B. Supplementary material

The following are the supplementary data to this paper:

Video S1
Video S2
Video S3
Video S4

Video S1. Movie of simulation with the discrete Lagrangian model from the top panel in Fig. 10. 1600 tumor cells and macrophages (in a 1:1 ratio) are initiated on the 2D surface, but can move in 3D. Macrophages are red and tumor cells are green. The EGF and CSF-1 gradient thresholds are 1%. The simulation is 48 h. Here the two cell types form an aggregate. A video clip is available online. Supplementary material related to this paper can be found online at http://dx.doi.org/10.1016/j.jtbi.2014.04.031
**Video S2.** Movie of simulation from Fig. 12. 1600 tumor cells and macrophages (in a 1:1 ratio) are initiated on the 2D surface, but can move in 3D. Macrophages are red and tumor cells are green. The simulation is 80 h. The EGF gradient threshold is 1% and the CSF-1 gradient threshold is 5%. Some of the macrophages are left outside the aggregate that forms resulting in a higher tumor cell to macrophage ratio. A video clip is available online. Supplementary material related to this paper can be found online at http://dx.doi.org/10.1016/j.jtbi.2014.04.031

**Video S3.** Movie of simulation where a micro-needle containing EGF is simulated (grey cylinder), Fig. 13 top panel. 400 tumor cells and macrophages (in a 1:1 ratio) are initiated on the 2D surface. Macrophages are red and tumor cells are green. Tumor cells sensitivity threshold to CSF-1 is 0.1%. 69 tumor cells and 37 macrophages are collected in the needle. $s_1 = 10^{-9}$ nM/(min cell) and $s_2 = 7 \times 10^{-8}$ nM/(min cell). A video clip is available online. Supplementary material related to this paper can be found online at http://dx.doi.org/10.1016/j.jtbi.2014.04.031
Video S4. Movie of simulation where a micro-needle containing EGF is simulated (grey cylinder), Fig. 13 bottom panel. 400 tumor cells and macrophages (in a 1:1 ratio) are initiated on the 2D surface. Macrophages are red and tumor cells are green. Tumor cells are less sensitive to a CSF-1 gradient than in the top panel of Fig. 13, 1% gradient threshold. The needle collects 59 tumor cells and 50 macrophages. $s_1 = 10^{-6} \text{nM/}(\text{min cell})$ and $s_2 = 7 \times 10^{-6} \text{nM/}(\text{min cell})$. A video clip is available online. Supplementary material related to this paper can be found online at http://dx.doi.org/10.1016/j.jtbi.2014.04.031

References