Basic ingredients for mathematical modeling of tumor growth
in vitro: Cooperative effects and search for space

F.H.S. Costa, M. Campos, O.E. Aiéllol, M.A.A. da Silvad

Departamento de Física, FFCLRP, Universidade de São Paulo, 14040-901 Ribeirão Preto, São Paulo, Brazil
b Departamento de Química e Ciências Ambientais, IBILCE, Universidade Estadual Paulista Júlio de Mesquita Filho, 15054-000 São José do Rio Preto, São Paulo, Brazil
c Departamento de Física Médica, UNIFEB, 14783-226 Barretos, São Paulo, Brazil
d Departamento de Física e Química, FCFRP, Universidade de São Paulo, 14040-903 Ribeirão Preto, São Paulo, Brazil

AUTHOR-HIGHLIGHTS

• We show that a basic requirement to simulate successfully the tumor growing in vitro is to adopt a sigmoidal growth rate.
• We use a different kind of dynamical Monte Carlo method, building the waiting times along the simulation.
• We have obtained non-Poissonian distributions for these waiting times.

ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 21 June 2013
Accepted 31 July 2013
Available online 14 August 2013

Keywords:
Tumor growth
Dynamical Monte Carlo
Mathematical modeling

ABSTRACT

Based on the literature data from HT-29 cell monolayers, we develop a model for its growth, analogous to an epidemic model, mixing local and global interactions. First, we propose and solve a deterministic equation for the progress of these colonies. Thus, we add a stochastic (local) interaction and simulate the evolution of an Eden-like aggregate by using dynamical Monte Carlo methods. The growth curves of both deterministic and stochastic models are in excellent agreement with the experimental observations. The waiting times distributions, generated via our stochastic model, allowed us to analyze the role of mesoscopic events. We obtain log-normal distributions in the initial stages of the growth and Gaussians at long times. We interpret these outcomes in the light of cellular division events: in the early stages, the phenomena are dependent each other in a multiplicative geometric-based process, and they are independent at long times. We conclude that the main ingredients for a good minimalist model of tumor growth, at mesoscopic level, are intrinsic cooperative mechanisms and competitive search for space.

1. Introduction

Mathematical modeling of biological systems, such as tumor growth, has an important role on in vitro (or in vivo) experiments concerning to formulate hypotheses about mechanisms and in suggesting new assays (Byrne, 2010). In fact, there is a growing interest in cancer modeling, since the scientific community begins to see it as a complex systems disease (Hornberg et al., 2006; Laubenbacher et al., 2009), which involves from genetic alterations (Hanahan and Weinberg, 2000) up to tissue aspects (Titz and Jeraj, 2008; Rejniak and McCawley, 2010). Regarding to clinical applications, one believes that the integration of imaging, treatment–response relationships, molecular basis, and predictive trials might speed up the development of more specific and more effective therapies (Byrne, 2010; Laubenbacher et al., 2009; Stewart and Li, 2007; Titz and Jeraj, 2008; Barazzuol et al., 2010; Kazmi et al., 2012; Román-Roamín and Torrez-Ruiz, 2012). Thus, we emphasize the importance of both mathematical and biological modeling and their uses in a complementary way (Byrne, 2010).

Tumor evolution is a complex process involving several phenomena at different scales (Preziosi, 2003). An approach for the growth may be done looking at mesoscopic events; e.g., cell–cell and cell–environment interactions, time interval between duplications, competition for space, formation or break of bonds that maintain the aggregate structure, and the temporal dynamics of the colonies size. To simulate such tumor progress, we may construct simple models just representing cells by its physical properties, despite their biological complexity (Drasdo et al., 2007). An important contribution of such systematizations (Block et al., 2007; Huergo et al., 2012), even if in two dimensions, is the classification of tumor growth patterns (Guiot et al., 2003),...
by generic mechanisms at individual cell level (migration, division, etc.), including molecular inter and intracellular regulation effects (Jiang et al., 2005), pressure effects (Brú and Casero, 2006) and evolution of cooperation (Alexrod et al., 2006). Also, one could use these models to identify cellular activities, which modified, would result in a maximal inhibition of multicellular evolution, and thus, point out potential therapeutic targets (Block et al., 2007; Katira et al., 2012). Brú and colleagues (Brú et al., 1998, 2003), in their investigation of pattern formation from several cell lines, highlighted the importance of the geometric structure and competition for space on the aggregate boundary. In recent work (Radszuweit et al., 2009), the authors search for simple and common mechanisms for tumor growth; through analysis of 2D and 3D models they suggested that single cell-based models in two-dimensions may describe well the general dynamics of its population.

In the search of mechanisms for tumor growth, by using simple models, we raised the following question: what ingredients are necessary to capture important features of tumor kinetics in the mesoscopic scale? Our belief is that cooperative effects and competitive search for space is the answer. In the next section we present the numerical method and the model used to simulate tumor growth; results and discussion appear within the third section, and in the last one we show the conclusions and point out our perspectives for future works.

2. Model and methods

We start our modeling approach, in the continuous limit, by fitting the experimental data (see Fig. 1) by using the following sigmoidal equation:

\[
\omega(t) = \frac{\alpha}{1 + \exp[\gamma(t-t_\gamma)]}
\]

with \(\omega(t) = \frac{\partial r(t)}{\partial t}\) being the mean radius rate.\(^1\) At early times the growth rate is lower, constant, and given by \(\alpha_0 = \alpha - \beta\). After a critical time \(t_\gamma\), the curve changes its behavior by going to another constant value \(\alpha\). The parameter \(\gamma\) determines how fast the rate changes from \(\alpha - \beta\) to \(\alpha\) \((\alpha > \beta)\). Thus, given the condition \(\omega(0) = \omega_0\), we can find the equation to the mean radius:

\[
r(t) = r_0 + \frac{\beta}{\gamma} \ln \left\{ \frac{\exp[\gamma(t-t_\gamma)] + 1}{\exp[\gamma t_\gamma] + 1} \right\} + \alpha t.
\]

Now, we introduce a discrete (minimalist) model using a lattice with \(M = L \times L\) sites, in which each site can only be in a tumor status \(T\) or in an empty status \(V\). We assume that the occupancy probability \(p_0\) of an empty site next to a tumor site carries the local and global information of the system at each instant; our global/local interaction is different from the one in the literature for epidemic models (Aiello et al., 2000; Aiello and da Silva, 2003; Cardy and Grassberger, 1985). There, they put the effects explicitly, while here, we bring them together. In this context, we assume that \(p_0\) comes directly from Eq. (1) by doing \(p_0 = p_0(t) = \omega(t)/\alpha\); consequently, we can write the transition rate for each empty site in the form \(g_q(t) \propto [1-(1-p_0)^{n_q}]\) (Cardy and Grassberger, 1985), where \(n_q\) is the number of neighbors with status \(T\) of an empty site labeled with index \(q\). Finally, we can write the transition probability per unit of time as

\[
g_q(t) = b \left\{ 1 - \left[ \frac{\beta}{\alpha} \left[ 1 + \exp[\gamma(t-t_\gamma)] \right]^{n_q} \right] \right\},
\]

where \(b\) is the frequency of new tumor sites in a colony. Here we consider the first and second nearest neighbors, i.e., \(0 \leq n_q \leq 2\). Also we consider that just one event occurs at each time interval \(\Delta t\), i.e., \(\Delta n_T = \Delta n_V = 1\). Thus, we can write the stochastic equation (Aiello and da Silva, 2003)

\[
d\frac{d}{dt} n_T(t) = \sum_q (g_q(t)) \cdot P_j(t) n_0^j,
\]

where \(\sum_q (\cdots)\) is the sum of all possible system configurations available at time \(t\); \(g_q(t) = \sum_{p=0}^{n_q} g_q(t) n_0^p\) represents the mesoscopic rate of the growth (an average over each configuration \(j\)); \(P_j(t)\) is the probability of finding the system in the state \(j\) at time \(t\); and \(n_0\) (from now on we will omit the configurational index \(j\) for all variables) is the total number of empty sites in the colony–medium interface; some of these sites may be inside the colony. The total number of lattice sites is \(M = n_T + n_V\), being \(n_V = n_0 + n_T\) the total number of empty sites, i.e., those \((n_V)\) which contribute to the increase of \(n_T\) (with \(n_V > 0\)), plus those \((n_T)\) that do not contribute (with \(n_V = 0\)). We neglect (explicitly) the cell death, migration and other process that could reduce the aggregate area, i.e., the transition \(T \rightarrow V\).

We solve Eq. (4) using the dynamical Monte Carlo method (DMC) approach (Aiello and da Silva, 2003). In the simulations, we estimate the average waiting time between two events with the expression

\[
\Delta t(\omega) = \frac{1}{\sum_q g_q(t)}
\]

The superscript \((\omega)\) denotes the average waiting time between the \((\omega - \Delta \omega)\)-th and the \((\omega)\)-th cell growth event. Finally, we use the following dynamical hierarchy (Aiello et al., 2000):

\[
H_q = \frac{g_q(t)}{\max g_q(t)} = \frac{1-(1-p_0)^{n_q}}{1-(1-p_0)^{n_T}}.
\]

where \(\max g_q(t)\) denotes the maximum value of \(g_q(t)\).

---

\(^1\) The derivative is obtained from the average slopes of adjacent points for each experimental data point.
Fig. 2. Temporal evolution of the mean radius of aggregates of the HT-29 cell line. We can see the good agreement between the experimental data, analytical Eq. (2) and simulation.

time: t→t+Δt^{(m)}; n_\text{f} increases of Δn_\text{f}, another mean radius is found, and the mesoscopic rate is updated (see Appendix A to the implementation of the mean radius and mesoscopic rate calculus). On the other hand, if H_\text{q}≤ξ, a new site is chosen and the process restart. At long times, we have g_\text{q}(t)→b, giving H_\text{q}=1; thus, the probability of transition will be the same of the Eden-A model (Jullien and Botet, 1985), i.e., 1/n_\text{r}. For early times, the behavior is not the same, because g_\text{q}(t) depends locally on the number of neighboring T-sites, so the chance of chooses and fill a site will be H_\text{q}/n_\text{r}.

3. Results and discussion

The sigmoidal growth rate ansatz allowed us to obtain Eq. (2) for the mean radius. Adjusting it to experimental data, we could find the values of the parameters α, β, γ and t, showed in Fig. 2, Eq. (1) points out three different regimes: the first one in which the growth rate is almost constant, equals to α–β; a second one, a transition regime, and the last one, where the growth rate is constant with value α. Suppose that all cells are synchronized and duplicate simultaneously, thus, the time to form a new layer can be given by the product of the number of the empty sites by the waiting time. However, as this does not occur (the cells are not synchronized), we multiply this by the average probability of occupation of an empty site, i.e., t_{\text{cycle}}=n_\text{r}Δt^{(m)}(H_\text{q}). This results that the average cell cycle time is 1/b. By adjusting the parameter b in the simulation to fit the experimental data we obtain 1/b ≈ 23.8 h, what is a good value, near to the expected 24 h (Tonkinson et al., 1999; Calabro-Jones et al., 1982). We believe that the apparent sigmoidal shape of the experimental data showed in Fig. 1 may result from cooperative effects at the growing perimeter of the colony. A possible cooperative mechanism is that the cells do not grow immediately after being plated in culture bottles; they need to become adherent to the plate surface for proliferate, i.e., there is a critical nucleus stabilized by cohesive forces favoring the adhesion of the cells to the plate surface. However, this hypothesis needs to be experimentally checked yet.

For study of the stochastic model, we used a lattice of L=500 sites. Since the number of the lattice side L and the diameter of a cell may be given by 1/L = d_0 = 10 μm (Drasdo and Hoehme, 2005). Initially one places a single cell in the lattice center. In Figs. 2 and 4, one takes the sample average from 200 runs, with estimated errors of about 1%, which makes the error bars smaller than the symbols of the measured quantities. We built the distributions of the average waiting times with 10^6 trajectories. We choose

\[ t = 60 \pm 10 \text{ h} \]
\[ t = 459 \pm 3 \text{ h} \]

...
1. To see the difference between fits with more details, we show the residuals of $\rho_g$ and $\rho_l$. With few cells, the log-normal curve was better, while in a big cluster, there is no much distinction between both.

Rigorously, for random processes having a Poisson distribution is required independence of events (Aiello et al., 2000; Aiello and da Silva, 2003; Fichtorn and Weinberg, 1991). Nevertheless, as seen before, log-normal and Gaussian curves fit well our data when using the average waiting times given by Eq. (5), reflecting $q_{\rho_g} \approx q_{\rho_l} \approx 1$. To see the difference between fits with more details, we show the residuals of $\rho_g$ and $\rho_l$. With few cells, the log-normal curve was better, while in a big cluster, there is no much distinction between both.

### Table 1

The adjusted parameters to $\rho_m$.

<table>
<thead>
<tr>
<th>Cells</th>
<th>25</th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_g$</td>
<td>42</td>
<td>48</td>
<td>114</td>
<td>340</td>
<td>974</td>
</tr>
<tr>
<td>$b_l$</td>
<td>35.7</td>
<td>47.8</td>
<td>113.9</td>
<td>339.5</td>
<td>947</td>
</tr>
<tr>
<td>$\langle \Delta t^{(m)} \rangle$</td>
<td>1.10</td>
<td>0.47</td>
<td>0.117</td>
<td>$1.62 \times 10^{-2}$</td>
<td>$4.56 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

The errors are less than 1%.
that there may have some dependency among the events. When
the colony has few cells, the behavior of \( \Delta t^{(m)} \) depends on the
empty site choice in a particular position, which may cause a log-
normal process. This emphasizes the importance of the local
interactions, at least in this initial stage. On the other hand, when
the colony is large, the waiting time becomes proportional to the
probability of choosing an empty site, this makes \( \Delta t^{(m)} \) to depend
negligibly on the position of the site chosen. However, we believe
that geometric details with the local interactions of the system
in vitro still have relevance in this region, especially to the
fractality of the aggregate (Drasdo et al., 2007; Block et al., 2007;
Brü et al., 1998, 2003). We could not reproduce the experimental
results using non-Poissonian waiting times distributions, which are
useful to describe the colony evolution. We evaluated its temporal
dynamics by means of computer simulations, being that all results
agreed among themselves and with the experimental data.

Using the DMC procedure (Aiello and da Silva, 2003), we found
non-Poissonian waiting times distributions, which are useful to
study the behavior of tumoral colonies. We showed that both
simulations, the Poissonian type and our approach, agreed. However,
we note that the above discussed process cannot be truly
Poissonian; it is just a numerical approach that gives the correct
solution for the master equation (Ålèllo et al., 2000; Fichhorn
and Weinberg, 1991). We built the distribution of waiting times for
several values of \( n_1 \), and we adjusted these data with log-normal
and Gaussian distributions, meaning that the events involved in the
processes are in some way dependent; we observe that log-
normal distribution, usually, fit better to our data than the
Gaussian ones. We found that the average waiting time, decays
with the number of cells given by the power law \( \langle \Delta t^{(m)} \rangle \propto n_1^{x} \).
The value of the exponent \( x \) may be an intrinsic feature of
monolayer growth, but more detailed studies are necessary to
clarify this result.

We believe that the basic mechanisms which make a minimalist
model works for monolayer tumor growth in vitro, in the meso-
scopic scale, are competitive search for empty spaces and intrinsic
cooperative mechanisms. These cooperative mechanisms may be
correlated with several factors, such as nutrients consumption and
adhesive/cohesive forces. In future work, we intend to do investiga-
tions in vitro to see how the number of cells varies with the mean
radius of the colonies. We, also, intend to verify experimentally the
lognormality of the waiting time distributions, and extend our model
to include cell deformation, cell cycle, nutrients and adhesion/cohesion
effects; we expect to reproduce naturally the sigmoidal rate behavior
with these additional ingredients.

**Acknowledgments**

F.H.S.C. is grateful to funding support from CAPES and we thank
FAPESP (Grant no. 2012/03823-5) for financial assistance.

**Appendix A. The mean radius and growth rate calculation**

Along the system evolution, we are interested in the mean
radius calculation, given by \( \langle r \rangle = \sqrt{2d \sigma_{r_{g}}} \), where \( d \)
is the cell diameter, and \( r_{g} \) is the gyration radius, \( r_{g} = \sqrt{(1/n_{t}) \sum_{i=1}^{n_{t}} |r_{i} - r_{cm}(t)|^{2}} \), being \( n_{t} \) the
number of cell, \( t \) the cell localization in the lattice, and \( r_{cm}(t) = (1/n_{t}) \sum_{i=1}^{n_{t}} r_{i} \), the center of
mass. We define the quantity \( S(t) = \sum_{i=1}^{n_{t}} |r_{i} - r_{cm}(t)|^{2} \) to use below.
When the system increases by just one cell in the time interval
\( \Delta t^{(m)} = \Delta t \), i.e., \( n_{t} \rightarrow n_{t} + 1 \), we can calculate a quantity \( \delta \) defined by

\[
\delta = \Delta r_{cm} = r_{cm}(t) - r_{cm}(t - \Delta t).
\]

To optimize the calculus of \( r_{g} \), instead of sweep all T-sites, we will
consider a function \( S(t) \) given by

\[
S(t) = S(t - \Delta t) + [r_{n_{t}} - r_{cm}(t)]^{2}.
\]

In this context, \( S(t - \Delta t) = \sum_{i=1}^{n_{t}-1} |r_{i} - r_{cm}(t - \Delta t)|^{2} \). Using Eq. (A.1)
one can rewrite Eq. (A.2) as

\[
S(t) = \sum_{i=1}^{n_{t}-1} |r_{i} - r_{cm}(t - \delta)|^{2} + [r_{n_{t}} - r_{cm}(t)]^{2}.
\]

After some algebraic manipulation we get

\[
S(t) = \sum_{i=1}^{n_{t}-1} |r_{i} - r_{cm}(t)|^{2} + [r_{n_{t}} - r_{cm}(t)]^{2} + 2\delta \cdot \sum_{i=1}^{n_{t}-1} |r_{i} - r_{cm}(t)| + \sum_{i=1}^{n_{t}} \delta^{2}.
\]

Considering that

\[
\sum_{i=1}^{n_{t}} |r_{i} - r_{cm}(t)|^{2} + [r_{n_{t}} - r_{cm}(t)]^{2} = \sum_{i=1}^{n_{t}} |r_{i} - r_{cm}(t)|^{2} = S(t),
\]

and, as by definition \( \sum_{i=1}^{n_{t}} |r_{i} - r_{cm}(t)| = 0 \), we obtain an alternative
form to \( S(t) \) from Eq. (A.4):

\[
S(t) = S(t) + 2\delta \cdot [r_{n_{t}} - r_{cm}(t)] - \delta^{2}(n_{t} - 1).
\]

This equation is useful due to the gain in CPU time, since we do not
need to sweep all T-sites at each update, as expected in \( r_{g} \)
definition; we can use just the current value of \( n_{t} \) to update \( r_{g} \).

Also in order of optimize the CPU time, in the waiting time
estimation (Eq. (5)) the growth rate can be replaced by

\[
\sum_{q=0}^{q_{m}} \sum_{n=1}^{n_{q}} \sum_{p_{q}} n_{p_{q}}[1-\left(1-p_{q}\right)^{q_{m}}],
\]

being \( n_{q} \) the number of empty sites with \( q \) tumoral neighbors. Note
that \( \sum_{n=1}^{n_{q}} n_{q} = n_{q} \); this relation is important when \( p_{q} \rightarrow 1 \). In this
way, the update is simplified sweeping only \( n_{\text{max}} \) elements, instead
of sweep all the \( q \) elements to find the growth rate.
References


