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# The universal growth rate behavior and regime transition in adherent cell colonies

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## HIGHLIGHTS

- We present an analysis of the growth rate of adhrent cell colonies.
- We show that five cell lineages share the same temporal behavior of the growth rate.
- Kinetic parameters in our model are estimated using the experimental data.

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## ABSTRACT

In this work, we used five cell lineages, cultivated in vitro, to show they follow a common functional form to the growth rate: a sigmoidal curve, suggesting that competition and cooperation (usual mechanisms for systems with this behavior) might be present. Both theoretical and experimental investigations, on the causes of this behavior, are challenging for the research field; since the sigmoidal form to the growth rate seems to absorb important properties of such systems, e.g., cell deformation and statistical interactions. We shed some light on this subject by showing how cell spreading affects the radius behavior of the growing colonies. Doing numerical time derivatives of the experimental data, we obtained the growth rates. Using reduced variables for the time and rates, we obtained the collapse of all colonies growth rates onto one curve with sigmoidal shape. This suggests a universal-type behavior, with regime transition related to a morphological transition of adherent cell colonies.



## 1. Introduction

Mathematical modeling of tumor growth has been an issue over decades, this has broken down the barriers between experimental, theoretical and computational sciences, and helped us to establish new connections between biology, physics and mathematics (Byrne, 2010). Especially in the last fifteen years, the search for universality classes has made this field of research highly fertile (Brú et al., 1998, 2003; Huergo et al., 2010, 2011, 2012). However, an alternative approach, paying attention to the radius growth rate of the colonies, could be used in the search for essential mechanisms that would rule the dynamics and the morphology of the aggregates of cancerous cells. In fact, the rate at which the number of cells increases or decreases has always been a central point used to evaluate the mechanisms involved in the growth of a population (Freyer and Sutherland, 1986), or the effectiveness of a specific treatment (Leith et al., 1993). Sometimes, the approach to the rate is done without taking into account the details that lead to the formation of aggregates, e.g., neglecting the cell to cell interactions (Dawson and Hillen, 2006).

Over the last 20 years, there has been an increasing interest in the study of interactions between cells and in the formation of monolayer aggregates. These studies are done, for example, to refine the margins of irradiation (Unkelbach et al., 2014); to verify possible tumor infiltrations in the healthy tissue (Poplawski et al., 2009); to quantify instabilities in the contour (Amar et al., 2011; Chatelain et al., 2011); changes in the cell cycle (Kim et al., 2014); or to identify known patterns (Brú et al., 1998, 2003; Huergo et al., 2011, 2012). In particular, in the study of monolayer cluster formation, when one does a modeling that includes the effects of proliferation and diffusion explicitly, a wide range of parameters can be chosen to fit the experimental data (Maini et al. 2004a,b). Some experimental work has been done to achieve clear rules for

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its determinations and reduce the multiplicity of choices (Simpson et al., 2013; Treolar et al., 2014).

An alternative approach to deal with aggregates is to propose phenomenological equations using effective growth rates (Drasdo and Hoëhme, 2005; Radszuweit et al., 2009). In the work of Costa et al. (2013) one proposes that the radius increasing rate has a sigmoidal shape. This could be a successful way to describe the time evolution of tumor colonies, since the experimental data and the theoretical modeling agreed quantitatively. Also, one has the advantage of describing this effective sigmoidal rate with continuous functions instead of step-like functions. Moreover, this type of rate behavior suggests the existence of a regime transition that we believe to be related with a morphological transition of cell colonies (Puliafito et al., 2012). Our goal in this work is to extend this approach to several cell lines, and show that the sigmoidal rate behavior is common, being an important intrinsic feature of this type of growth. Also, we discuss the possible mechanisms that may generate this type of behavior ubiquitously present in biochemical reactions, i.e., cooperative and competitive mechanisms.

## 2. Model and methods

### 2.1. In vitro experiments and image processing

We cultivated HeLa (human cervical carcinoma), HCT-115 (human colorectal adenocarcinoma),[1] B16–F10 (mouse melanoma)[2] and NIH-HN-13 (human head and neck squamous carcinoma) cells (passage 2–20). Also, we used data from HT-29 (human colon carcinoma) cell lineage (Brú et al., 2003). Cells were plated at low density (25–50 cells/ml) in corning culture flasks with a 25 $cm^2$ growth area. For HeLa and NIH-HN-13 cell lines we used 4 ml of DMEM medium containing 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin; for HCT-115 and B16–F10 cell lines we used 4 ml of RPMI-1640 medium with the same amount of supplements mentioned above. Cells were maintained in an atmosphere of 5% $CO_2$ and 37 °C. During the experiment the whole culture medium was carefully replaced every 3 days.

After 24 h of culture, several colonies with at least two cells were selected and imaged regularly with magnifications of 10× and 20× using two inverted microscopes: one with phase-contrast (Olympus IX-71, to image HeLa, HCT-115 and NIH-HN 13 cells) at the Centro de Pesquisas em Oncologia Molecular (CEPOM), Hospital de Câncer de Barretos-SP-Brazil, and the other equipped with DIC (Nikon Ti-Eclipse, to image B16–F10 cells) at the Laboratório de Química Analítica, Grupo de Física Biológica, Departamento de Física e Química, FCFRP/USP-SP-Brazil. Photographs were scanned with a resolution of 1 μm/pixel (10×) and 0.5 μm/pixel (20×) (IX-71), and, 0.64 μm/pixel (10×) and 0.32 μm/pixel (20×) (Ti-Eclipse). The colonies' profiles were hand-traced with the aid of *ImageJ*[3] and *CellSens* 2.0 software and the radius measurements of these profiles were done with in-house computer software. When necessary, the entire image of each colony resulted from the composition of partial images.

With our in-house software tool, we calculated the mean radius for the 10×-profiles using the relation (Costa et al., 2013) $r(t)=\sqrt{2}d_0 r_g$; where $d_0$ is the resolution in μm/pixel and $r_g=\sqrt{(1/n_p)\sum_{p=1}^{n_p}[\vec{r}_p-\vec{r}_{cm}(t)]^2}$ is the radius of gyration, obtained with the pixel's positions $\vec{r}_p$, the number of pixels $n_p$, and the center of mass $\vec{r}_{cm}(t)=(1/n_p)\sum_{p=1}^{n_p}\vec{r}_p$. Each growing colony was followed until reaching the border of a neighboring colony, sometimes taking approximately 330 h to have contact. For magnifications of 20× we manually counted the number of cells in the samples.

### 2.2. Mathematical model

As presented in previous work (Costa et al., 2013), we assume an effective rate described by the sigmoidal function:

$$\omega(t)=\frac{d}{dt}r(t)=\alpha-\frac{\beta}{1+\exp[\gamma(t-t_c)]}. \tag{1}$$

Initially, the growth is roughly linear with a constant rate $\omega_0\equiv\omega(t)\approx\alpha-\beta$. After a critical time $t_c$ (note that $\omega(t_c)=\alpha-\beta/2$), the curve changes its behavior going to a constant rate $\alpha$, where $\gamma$ is a parameter that determines how fast the rate evolves from $\omega_0$ to $\alpha$. Now, we can define the reduced variables for the rate and time, respectively, as follows:

$$\omega_c=\frac{[(\omega/\alpha)-1]}{\Gamma}, \tag{2}$$

and

$$\tau=\gamma(t-t_c), \tag{3}$$

with $\Gamma=\beta/\alpha$. After some algebraic manipulations with these variables, by substituting in Eq. (1), we obtain

$$\omega_c=-\frac{1}{1+\exp(\tau)}. \tag{4}$$

Fig. 1 illustrates the biological meaning of the four parameters in Eq. (1), by giving a pictorial view of a growing colony in five different instants.

Thus, despite the different parameters $\alpha$, $\beta$, $\gamma$ and $t_c$, intrinsic for each cell lineage, the reduced rate and time allow us to collapse the data onto a single sigmoidal-like growth rate curve. Therefore, we could fit the experimental data for the mean radius of the colonies with the function

$$r(t)=r_0+\frac{\beta}{\gamma}\ln\left\{\frac{\exp[-\gamma(t-t_c)]+1}{\exp(\gamma t_c)+1}\right\}+\alpha t, \tag{5}$$

which is the solution of Eq. (1) with the condition $r(0)=r_0\equiv 5$ μm (Drasdo and Hoëhme, 2005). For the curve fitting,[4] we did a shift in the data of the mean radius, for different colonies, of each cell line, following a similar approach of Brú et al. (1998) and Puliafito et al. (2012). There, the authors did shifts in the growth curves to coincide with the initial radius at $t=0$ (Brú et al.), and at the beginning of the morphological transition (Puliafito et al.). Here, we do shifts and fittings to build the growth curves, using the following scheme:

(i) make a data fitting for each individual colony with the function given by Eq. (5);
(ii) choose the best curve from the fittings of step (i) – that one with the smallest errors in the parameters – to use as a *guide-curve*;
(iii) minimize the sum of the residuals, between the guide-curve and the experimental data, of each individual colony, by doing temporal shifts and updating the colonies data;
(iv) put all final data together (including those used to build the guide curve), and make the fitting to obtain the values of the parameters $\alpha$, $\beta$, $\gamma$ and $t_c$.

## 3. Results

Fig. 2 shows the growth curves and the parameters of the sigmoidal rate (see Eq. (1)). For B16–F10 cells we found $\alpha=6.2\pm0.4$ μm/h. Also, we averaged the parameters obtained for each individual colony (recipe in the step (i) of the shift-fit scheme),

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[4] To see the data the reader is referred to the Online Supplementary Material.