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Distinguishing between mechanisms of cell aggregation using pair-correlation functions

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AUTHOR-HIGHLIGHTS

- Formation of cell aggregates is modelled with an agent-based model.
- Model includes cell proliferation and cell to cell attraction mechanisms.
- Pair-correlation function can identify the mechanisms that produce cell aggregation.
- Analysis of experimental images validates theoretical methods developed.

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ABSTRACT

Many cell types form clumps or aggregates when cultured *in vitro* through a variety of mechanisms including rapid cell proliferation, chemotaxis, or direct cell-to-cell contact. In this paper we develop an agent-based model to explore the formation of aggregates in cultures where cells are initially distributed uniformly, at random, on a two-dimensional substrate. Our model includes unbiased random cell motion, together with two mechanisms which can produce cell aggregates: (i) rapid cell proliferation and (ii) a biased cell motility mechanism where cells can sense other cells within a finite range, and will tend to move towards areas with higher numbers of cells. We then introduce a pair-correlation function which allows us to quantify aspects of the spatial patterns produced by our agent-based model. In particular, these pair-correlation functions are able to detect differences between domains populated uniformly at random (*i.e.* at the exclusion complete spatial randomness (ECSR) state) and those where the proliferation and biased motion rules have been employed – even when such differences are not obvious to the naked eye. The pair-correlation function can also detect the emergence of a characteristic inter-aggregate distance which occurs when the biased motion mechanism is dominant, and is not observed when cell proliferation is the main mechanism of aggregate formation. This suggests that applying the pair-correlation function to experimental images of cell aggregates may provide information about the mechanism associated with observed aggregates. As a proof of concept, we perform such analysis for images of cancer cell aggregates, which are known to be associated with rapid proliferation. The results of our analysis are consistent with the predictions of the proliferation-based simulations, which supports the potential usefulness of pair correlation functions for providing insight into the mechanisms of aggregate formation.

1. Introduction

The formation of clusters or aggregates is a ubiquitous phenomenon in cell biology; examples include cultures of *myxobacteria*, the slime mould *dictyostelium*, and many other cell types grown *in vitro* for cancer research, studies in developmental biology, or applications in tissue engineering (Alber et al., 2004; Binder et al., 2011; Green et al., 2010; Hofer et al., 1995; Savill and Sherratt, 2003; Painter and Sherratt, 2003; Thomas et al., 2006; Vasiev and Weijer, 2003). These aggregates can be produced by rapid cell proliferation (Simpson et al., 2013a) (as are, for example, those shown in Fig. 1(a)), or by other mechanisms involving attractive cell-to-cell interactions, such as chemotaxis, or direct physical contact, as is the case for those shown in Fig. 1(b) (Green et al., 2010; Thomas et al., 2006). Identifying the mechanism of aggregate formation in a particular case provides us with a

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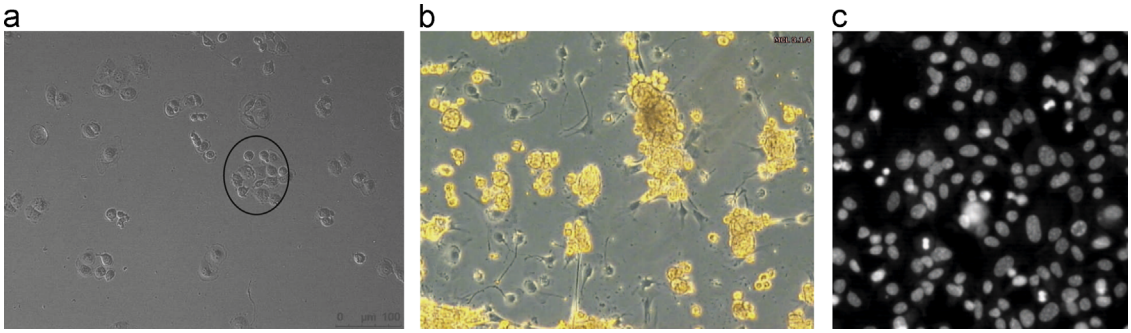

Fig. 1. Spatial patterning observed in images from several *in vitro* experiments. (a) Aggregates of MBA MD 231 breast cancer cells. (b) Aggregates of liver cells (reprinted from Thomas et al., 2006, with permission from Eur. Cells Mater.). (c) Uniform distribution of 3T3 fibroblast cells.

fundamental understanding of the underlying processes, and can also be of practical importance in, for example, optimising culture conditions to promote cell viability and functionality. In some cases, it may be possible to identify the mechanism of aggregate formation simply by observing the cells in culture – *e.g.* one can observe the physical contact between co-cultured hepatocytes and stellate cells using time-lapse video (Thomas et al., 2006). Unfortunately, this is not always possible since some observations are made at one point in time only. A complicating factor is that nearly all cell types undergo some degree of unbiased random motion. If a proliferation or attraction mechanism is absent, this type of unbiased motion leads to a uniform cell distribution and no spatial patterning will be observed (as in the case of the mouse cells shown in Fig. 1(c)). However, even when aggregating mechanisms are present, unbiased cell motion can obscure the details meaning that it is not obvious how to assess and measure the aggregation mechanism. Thus a means of analysing spatial patterns that is highly sensitive to the presence of non-random arrangements of cells, and able to provide information regarding the mechanism that drives aggregate formation, would be of considerable practical use.

In this study, we develop agent based models (Binder et al., 2011; Binder and Landman, 2009; Codling et al., 2008; Simpson et al., 2007) to analyse two common mechanisms of aggregate formation, each of which includes a component of random motion. We consider both a cell proliferation mechanism and a biased cell motion mechanism, in which cells detect the presence of others within a certain spatial range and attempt to move towards them. The latter mechanism is a generic representation of a variety of attractive interactions between cells, including direct physical contact and chemotaxis. We show that when the agents experience a sufficiently large amount of random motion it is difficult to distinguish the spatial patterns produced by either mechanism from cells distributed uniformly at random by visual inspection alone. This motivates us to introduce a modified pair-correlation function (Binder and Simpson, 2013; Cardarelli and Gratton, 2010; Dieckmann et al., 2000; Law et al., 2003; Young et al., 2001) to provide a more precise means of characterising these spatial patterns.

The pair-correlation function is a summary statistic that provides a quantitative measure of spatial patterning. The function is derived by normalising the counts of the pair-distances between pairs of agents in the domain. The normalisation term ensures that the expected value of the pair-correlation function is unity at all pair-distances for domains that are populated uniformly at random – termed the exclusion complete spatial randomness (ECSR) state (Binder and Landman, 2011; Binder and Simpson, 2013; Dieckmann et al., 2000; Diggle, 1983; Hackett-Jones et al., 2012). We show that the pair correlation function is sufficiently sensitive to distinguish the ECSR state from the patterns resulting from either of our cluster-forming mechanisms, even when no difference is detectable by eye. The pair-correlation function is also shown to provide important quantitative information on the spatial patterning, such as the multiple length scales of aggregation and segregation (Binder and Simpson, 2013; Dieckmann et al., 2000), and the size of aggregates.

In cases where there is little random motion, we can clearly observe from visual inspection of our simulations using the biased motion rule the emergence of a characteristic separation distance between aggregates, which is not observed when aggregates are formed by rapid cell proliferation. This difference is easily distinguished when comparing the pair correlation functions for the two types of simulation results. Significantly, we find that this difference in the pair correlation functions is still clearly evident even when the degree of unbiased motion is increased to a level such that it is impossible to distinguish any difference in the simulation results by eye. This demonstrates the potential utility of the pair-correlation function to identify and quantify mechanisms of aggregation in cell-based experiments.

Given the promising results from our simulation data, as a proof of concept, we apply our technique to experimental images of the cancer cells in Fig. 1(a). This cell type is known to form aggregates as a result of rapid cell proliferation (Simpson et al., 2013a). We process the experimental images, rescaling the cell location and cell area data so that it can be approximately represented on a non-dimensional integer lattice in a similar manner to the simulation results. Evaluating the pair-correlation function for this dimensionless data set we obtain a signal that is qualitatively similar to that produced by the proliferation mechanism in the agent-based simulations. This suggests, as expected, that these aggregates are formed predominately by cell proliferation.

2. Agent-based model

We consider the situation, relevant to many types of *in vitro* cell culture, in which cells are seeded onto a two-dimensional surface. It is assumed that initially the cells are distributed uniformly at random within this domain. We develop an agent-based model, discrete in time and space (Binder and Landman, 2009; Simpson et al., 2007), to simulate two mechanisms of aggregate formation: biased cell motion and cell proliferation, as outlined above. In both cases, cells are assumed to undergo a variable degree of unbiased random motion.

We define a two-dimensional domain, consisting of an integer lattice with unit spacing. We assume that this domain represents part of a larger region containing cells – for example, the region of a culture well within the field of view of microscope; we thus impose periodic boundary conditions in both the x- and