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Are *in vitro* estimates of cell diffusivity and cell proliferation rate sensitive to assay geometry?

Katrina K. Treloar [a,b], Matthew J. Simpson [a,b,*], D.L. Sean McElwain [b], Ruth E. Baker [c]

[a] Mathematical Sciences, Queensland University of Technology (QUT), Brisbane, Australia
[b] Tissue Repair and Regeneration Program, Institute of Health and Biomedical Innovation, QUT, Brisbane, Australia
[c] Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, United Kingdom

HIGHLIGHTS

- Spread of cell populations in two distinct *in vitro* assay geometries is analysed.
- Discrete and continuum models are compared to experimental results.
- Geometry of *in vitro* assay affects estimates of cell diffusivity by up to 50%.
- Cell proliferation rate estimates vary by up to 30% depending on assay geometry.
- Parameterised models accurately predict behaviour of spreading cell populations.

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ABSTRACT

Cells respond to various biochemical and physical cues during wound-healing and tumour progression. *in vitro* assays used to study these processes are typically conducted in one particular geometry and it is unclear how the assay geometry affects the capacity of cell populations to spread, or whether the relevant mechanisms, such as cell motility and cell proliferation, are somehow sensitive to the geometry of the assay. In this work we use a circular barrier assay to characterise the spreading of cell populations in two different geometries. Assay 1 describes a tumour-like geometry where a cell population spreads outwards into an open space. Assay 2 describes a wound-like geometry where a cell population spreads inwards to close a void. We use a combination of discrete and continuum mathematical models and automated image processing methods to obtain independent estimates of the effective cell diffusivity, D, and the effective cell proliferation rate, λ. Using our parameterised mathematical model we confirm that our estimates of D and λ accurately predict the time-evolution of the location of the leading edge and the cell density profiles for both assay 1 and assay 2. Our work suggests that the effective cell diffusivity is up to 50% lower for assay 2 compared to assay 1, whereas the effective cell proliferation rate is up to 30% lower for assay 2 compared to assay 1.

1. Introduction

Cell migration and cell proliferation are essential mechanisms that drive wound-healing and tumour progression (Clark, 1996; Geho et al., 2005; Martin, 1997; Weinberg, 2006; Woodhouse et al., 1997). During these processes, cells sense and respond to various biochemical and physical cues (Ashby and Zijlstra, 2012; Brock et al., 2003; Kilian et al., 2010; Lutolf and Hubbell, 2005; Vogel and Sheetz, 2006). Although the role of biochemical cues has been widely explored, it remains relatively unclear how physical cues, such as the local geometry, affect the capacity of cell populations to spread (Ashby and Zijlstra, 2012; Brock et al., 2003; Kilian et al., 2010; Lutolf and Hubbell, 2005; Vogel and Sheetz, 2006).

Wound-healing and tumour progression are often studied in the same context since the mechanisms that drive these processes are thought to be similar (Weinberg, 2006; Coussens and Werb, 2002; Chang et al., 2004; Friedl and Gilmour, 2009; Schafer and Werner, 2008). Despite their similarities, these processes have distinct geometries: (i) during wound-healing, cell populations spread inwards to close the wound void, and (ii) during tumour

* Corresponding author at: Mathematical Sciences, Queensland University of Technology (QUT), Brisbane, Australia. Tel.: +617 3138 5241; fax: +617 3138 2310.
E-mail address: matthew.simpson@qut.edu.au (M.J. Simpson).

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progression, cell populations spread outwards causing the tumour to expand (Weinberg, 2006; Ashby and Zijlstra, 2012).

Cell-based assays are commonly used to quantify the capacity of cell populations to spread *in vitro* (Ashby and Zijlstra, 2012; Kramer et al., 2013; Decaestecker et al., 2007; Kam et al., 2008, 2009; Valster et al., 2005). Several types of assays have been developed to investigate cell population spreading in two and three dimensions including Transwell, scratch, exclusion zone and spheroid assays (Ashby and Zijlstra, 2012; Kramer et al., 2013; Decaestecker et al., 2007; Valster et al., 2005). While these assays have been used to study the behaviour of various cell lines *in vitro*, most studies neglect to explicitly consider the role of geometry when conducting or interpreting these assays and it is unclear how results obtained for one particular geometry translate into another (Ashby and Zijlstra, 2012; Kramer et al., 2013; Decaestecker et al., 2007; Valster et al., 2005). Recent work using microfabrication methods focused on creating various-sized channels through which cells could migrate, with the observation that the speed of the leading edge of the cell population depends on the channel width (Vedula et al., 2012). Therefore, it seems reasonable to assume that assay geometry could play a role in determining the rate at which cell populations spread.

An alternative approach to understand how differences in geometry affect cell population spreading is to conduct a two-dimensional cell spreading assay where the direction of the spreading is intentionally varied. In this work, we will consider two types of assays:

Assay 1: This is a tumour-like assay initialised by placing cells inside a barrier, which is then lifted, allowing the population to spread outwards (Ashby and Zijlstra, 2012; Kramer et al., 2013).

Assay 2: This is a wound-like assay initiated by placing cells outside a barrier, which is then lifted, allowing the population to spread inwards (Ashby and Zijlstra, 2012; Kramer et al., 2013).

Without analysing any experimental data it is unclear whether a population of otherwise identical cells will exhibit different rates of spreading in the geometry of assay 1 compared to the geometry of assay 2.

A circular barrier assay can be used to study both assay 1 and assay 2 geometries, by initially placing the cells either inside or outside the barrier, which is then lifted to initiate the cell spreading (Ashby and Zijlstra, 2012; Kramer et al., 2013; Simpson et al., 2013; Treloar and Simpson, 2013; Van Horssen and Ten Hagen, 2010). Barrier assays are thought to be more reproducible than traditional mechanical wounding assays, such as scratch assays, as they do not damage the cell monolayer (Van Horssen and Ten Hagen, 2010; Gough et al., 2011). In this work, we will consider the spreading of cell populations in a barrier assay that are driven by combinations of motility and proliferation.

The standard continuum mathematical model used to describe how a population of motile and proliferative cells spread in two dimensions is related to the Fisher–Kolmogorov equation, and is given by

$$\frac{\partial \overline{c}}{\partial t} = D\nabla^2\overline{c} + \lambda\overline{c}\left(1-\frac{\overline{c}}{K}\right), \tag{1}$$

where $\overline{c}(x,y,t)$ [cells/L^2] is the dimensional cell density, D [L^2/T] is the cell diffusivity (random motility coefficient), λ [/T] is the cell proliferation rate and K [cells/L^2] is the carrying-capacity density (Murray, 2002; Sherratt and Murray, 1990; Swanson et al., 2003; Maini et al. 2004a,b; Sengers et al., 2007; Cai et al., 2007). Physical dimensions relevant to *in vitro* cell biology assays are μm and hours for L and T, respectively. Discrete random walk-based models which are related to Eq. (1) can also be used to study cell population spreading. Discrete models allow us to visualise the biological spreading process in a way that is directly comparable with experimental results (Simpson et al., 2013; Anderson et al., 2007; Anderson and Chaplain, 1998; Aubert et al., 2006; Deroulers et al., 2009; Codling et al., 2008; Simpson et al., 2010; Turner and Sherratt, 2002; Turner et al., 2004; McDougall et al., 2012). For example, snapshots from a discrete model showing the location of individual agents in the population can be easily compared to experimental images that show the location of individual cells in the population (Simpson et al., 2013; Treloar et al., 2013).

Previous studies have used Eq. (1) to estimate D and λ from experimental observations with the additional implicit assumption that these estimates could be relevant when considering the same cell population spreading in a different geometry. This standard assumption implies that estimates of D and λ obtained by calibrating Eq. (1) to observations in one particular geometry could be used to accurately predict the spreading of the same cell population, under the same experimental conditions, in a different geometry. However, from a biological point of view, it seems reasonable to anticipate that cell populations could respond differently under different circumstances. This means that our estimates of D and λ in Eq. (1) might be different when calibrating this model to different experimental conditions. For this reason we will refer to estimates of D as the *effective cell diffusivity* and our estimates of λ as the *effective cell proliferation rate*, thereby making it explicit that we are allowing for the possibility that these estimates could depend on the specific details for the experiment from which they are estimated.

In this work, we use a combined experimental and mathematical modelling approach to investigate how the two-dimensional spreading of a fibroblast cell population is influenced by the assay geometry. In particular, we address the following questions:

1. Do estimates of the effective cell diffusivity, D, depend on the geometry of the assay?
2. Do estimates of the effective cell proliferation rate, λ, depend on the geometry of the assay?
3. Does the geometry of the assay affect the rate at which the leading edge of the cell population moves?
4. Are the cell density profiles through the spreading cell population sensitive to changes in the geometry of the assay?

To answer these questions, we conduct several circular barrier experiments using assay 1 and assay 2 geometries. For both assay geometries we independently estimate the effective cell diffusivity, D, using experiments where cell proliferation is suppressed. The effective proliferation rate, λ, is then separately estimated using experiments where proliferation is not suppressed. To ensure that our estimates of D and λ accurately predict the position of the leading edge of the spreading population as well as the cell density profile throughout the spreading cell population we compare predictions of the parameterised mathematical model with experimental measurements. In summary, our results indicate that estimates of D and λ appear to depend on the assay geometry, with D being more sensitive than λ.

2. Experimental methods

2.1. Circular barrier assay

Fig. 1 shows a schematic diagram of the two barrier assay geometries considered in this work. To perform these assays metal-silicone barriers (Aix Scientifics, Germany) were cleaned, sterilised, dried and placed in the centre of the wells of a 24-well tissue culture plate. The wells in the tissue culture plate have a diameter of 15,600 μm. The barrier has an approximate radius of 3000 μm inside the silicone tip (located at the end of the barrier) and 4000 μm outside the silicone tip.

Experiments were conducted with fibroblast cells (supplementary material) where, in some cases the spreading was driven by