Quantifying the role of different surface coatings in experimental models of wound healing

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Abstract

In vitro surface coatings are widely used to mimic the role of extracellular matrix in the *in vivo* environment. Different effects are reported for different surface coatings, however, some of these results are inconsistent across the literature. To explore the role of different surface coatings, we use a new modified stopper-based wound-healing assay, called a *stopper assay*, with two commonly used surface coatings: gelatin and poly-L-lysine (PLL). Our experimental data show the gap width decreases faster with the gelatin and PLL coatings. Similarly, the number of cells in certain subregions increases faster with these coatings. Unfortunately, neither of these observations provides definitive mechanistic insight into the role of the coatings. To provide such insight we calibrate the solution of the Fisher-Kolmogorov model to match the experimental data. Our parameter estimates indicate that both coatings significantly increase cell motility without affecting cell proliferation.

Key words: Wound healing assay; Stopper assay; Surface coating; Fisher-Kolmogorov; Reaction-diffusion; Transport

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1 Introduction

In vitro surface coatings are used as a fundamental experimental approach to mimic and study the effect of the extracellular matrix (ECM) in the *in vivo* environment. It is important to include effects of the ECM in experimental studies because the ECM interacts with cells both physically and chemically, affects cell behaviour, and plays an important role in biological processes such as tissue development and cancer progression (Hay 2013; Horiguchi et al., 2012; Screen et al., 2015; Vedula et al., 2013). Popular choices of coating reagents include gelatin, poly-L-lysine (PLL), collagen, and fibronectin (Liberio et al., 2014; McCarthy et al., 1983; McIntosh et al., 1988). The precise role of surface coatings, however, remains unclear since experimental observations are inconsistent. For example, gelatin is thought to increase both cell motility and proliferation (McCarthy et al., 1983; McIntosh et al., 1988), while PLL can have the opposite effects (Liberio et al., 2014). In contrast, other studies suggest that PLL may have no impact on cell motility or proliferation (Fischer et al., 2007; Rangappa et al., 2000).

Among various types of *in vitro* experiments, wound-healing assays are widely used to study cell migration and cell proliferation under different conditions including the use of different surface coatings (Ascione et al., 2017; Liberio et al., 2014; Tremel et al., 2009). Wound-healing assays are initiated by creating a scratch in a monolayer of cells, followed by the observation of how the remaining cells migrate and proliferate to close the gap over time. This type of assay can be used to provide quantitative insight by measuring rates of the gap closure or measuring the increase in the numbers of cells in particular subregions of the experiment (Johnston et al., 2014; Liberio et al., 2014; McCarthy et al., 1983; Treloar and Simpson, 2013). However, these measurements alone cannot tease apart the intricate interplay between the effects of cell migration and cell proliferation that can sometimes lead to surprising results. For example, Barrandon and Green (1987) measure the temporal change in the numbers of cells in certain *in vitro* colonies and note a dramatic increase in cell numbers

when TGF-α is included. This increase in cell number is due to the interplay of cell migration and cell proliferation, since TGF-α promotes migration, which then in turn provides space for the cells to proliferate (Barrandon and Green, 1987).

Mathematical models have been applied to mimic both *in vitro* and *in vivo* experiments to test biological hypotheses and to predict experimental outcomes (Jin et al., 2018a; Jove et al., 2019; Nardini et al., 2016; Sheardown and Cheng, 1996; Tam et al., 2019; Villella et al., 2019). One of the most commonly used models to study collective cell migration is the Fisher-Kolmogorov model (Fisher, 1937; Maini et al., 2004; Sherratt and Murray, 1990; Simpson et al., 2006). The Fisher-Kolmogorov model describes the interplay between cell migration and cell proliferation by assuming (i) cells migrate according to undirected linear diffusion, and (ii) cells proliferate logistically to a carrying capacity density. In previous studies, the Fisher-Kolmogorov model has been calibrated to match experimental data from *in vitro* wound-healing assays, and has been used to provide mechanistic insight into the role of the initial seeding density, the wound geometry, and the shape of the migrating front (Jin et al., 2016; Jin et al., 2018b; Sengers et al., 2007).

In this work we apply a new modified stopper-based *in vitro* wound-healing assay, called a *stopper assay*, to explore the role of two widely used surface coatings: gelatin and PLL. Compared to other commercial experimental apparatus (Liberio et al., 2014; Qi et al., 2019), one important feature of the stopper assays is that we can study two different coating conditions simultaneously in one experimental well. This allows us to make direct comparisons of how different coatings affect gap closure in the same field-of-view without being concerned by potential differences caused by monitoring different experimental wells. Our experimental data describes the gap width and the increase in the numbers of cells in particular subregions in the experiment. This data indicates that the gap closes faster, and the cell population increases faster in the gelatin- and PLL-coated regions. By calibrating the Fisher-Kolmogorov model to match the experimental data, we find that both gelatin and PLL

coatings significantly increase the cell diffusivity without affecting the rate of cell proliferation.

2 Methods

2.1 Experimental methods

Stopper assays are performed in six–well experimental plates (Corning, USA), in which each experimental well has a diameter of 35 mm. Schematics of the stopper assays are illustrated in Figure 1. The lower semicircular surface of the experimental well is covered with tape (8018, 3M) to form the uncoated control area (Figure 1(a)). For the surface coating, sterile 0.1% (w/w) gelatin (Sigma, USA) or 0.1 μg/mL poly-L-lysine (Sigma, USA) prepared in 1X phosphate-buffered saline (PBS) is added to the well and incubated at 37 °C for 30 min. After the excess liquid is aspirated completely, the tape is removed, with only the upper semicircular surface of the well coated (Figure 1(a)). In the remainder of this work, we refer to the upper and lower semicircular surfaces of the experimental well as the *upper half* and the *lower half*, respectively.

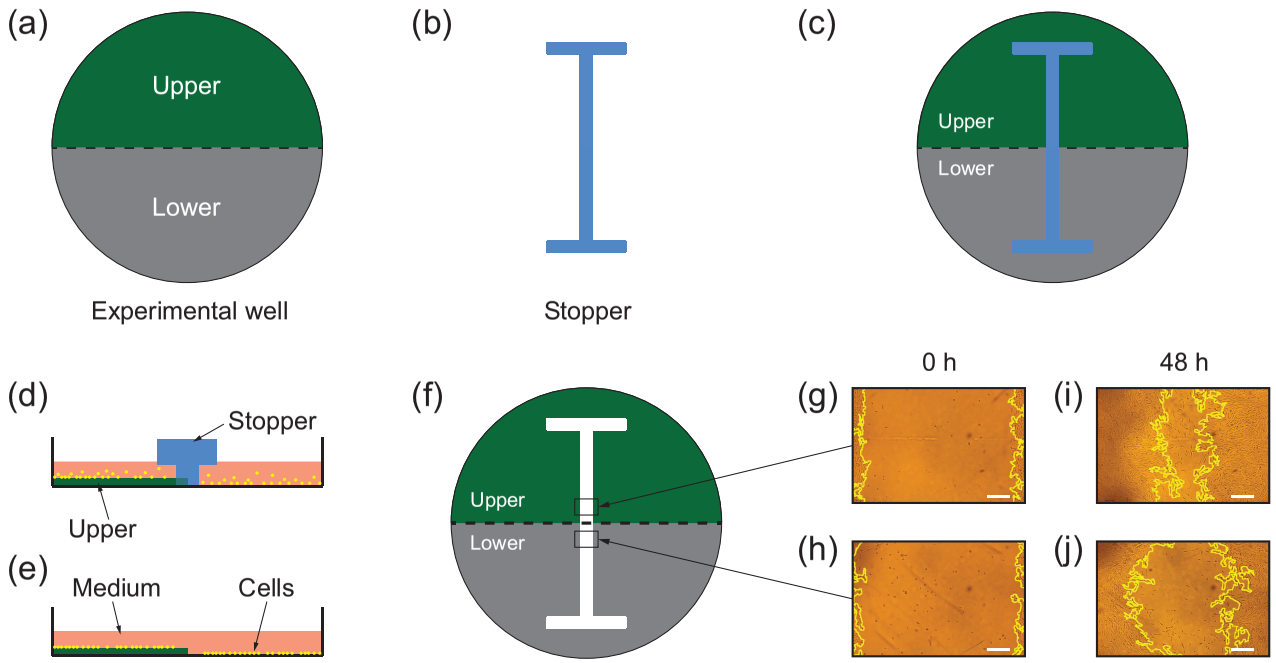

Fig. 1. **Illustrations of *in vitro* stopper assays.** (a) Experimental well in which the upper half is coated. (b) A stopper for creating the cell-free region. (c) Experimental well with a stopper placed in the middle. (d) Section of the stopper assay with a stopper in place. The thick green line indicates the upper half and the yellow dots indicate cells. (e) Section of the stopper assay after a cell monolayer is grown and the stopper is removed. (f) An illustration of how the data are retrieved from the stopper assay. The white space indicates the initially vacant area. The black rectangles correspond to regions where two experimental images are taken. (g)–(h) Experimental images taken at $t = 0$ h in the indicated upper and lower halves, shown in (f). (i)–(j) Experimental images taken at $t = 48$ h in the indicated upper and lower halves. The yellow line indicates edges of the gap. The scale bar corresponds to 500 μm.

We perform the experiments with the mouse embryonic fibroblast cell line NIH 3T3, purchased from the Bioresource Collection and Research Center (BCRC), Taiwan. The cell culture medium consists of Dulbecco's Modified Eagle's medium (DMEM, Gibco, USA) and 10% calf serum (CS, Invitrogen, USA). Although the calf serum can adhere to the surface of the experimental wells and potentially interact with surface coatings, many studies that use calf serum do not consider this to be an explicit coating treatment (Gospodarowicz et al., 1983; Liberio et al., 2014; Rangappa et al., 2000). Cells are incubated in cell culture flasks (Corning, USA) under 5% CO_2 at 37 °C.

A customized I-shaped stopper (Figure 1(b)), made of polydimethylsiloxane (PDMS, Dow Corning Sylgard^{TM} 184 Silicone Elastomer, USA), is placed into the experimental well so that it is perpendicular to the boundary of the upper and lower halves (Figure 1(c)). Approximately 2.5×10^5 cells are seeded uniformly into the six–well experimental plates and incubated overnight (Figure 1(d)). The stopper is then removed to create an initial gap (Figure 1(e)). After removing the stopper, cells are free to migrate and proliferate, eventually leading to the closure of the initially vacant space in both the upper and lower halves (Figure 1(g)-(i)). The gap width and the number of cells in certain subregions are measured at five equally-spaced time points: $t = 0, 12, 24, 36$, and 48 h. Each experiment is performed three times so that we can extract data from each replicate and average the data across the replicates. In this work we use data describing individual experimental replicates as well as the averaged data. To distinguish these two types of the data, we use superscripts on certain variables to indicate the individual replicate number, and we use the variables with a tilde to indicate the averaged data.

Before presenting our experimental and modelling results, we now describe some of the terminology we use to describe the experimental protocol and experimental data. Some experiments do not involve any coating at all, and we refer to these experiments as *control* experiments. Other experiments involve applying different coatings to the experimental well, of which the upper half is coated with gelatin or PLL and the lower half is uncoated (Figure 1(f)). This

means that we have two different types of control assays: one type corresponds to the control experiments, and the other type corresponds to the uncoated regions in the coating experiments. In Section 3.1 we plot the average data for the control experiment as well as the average data in the upper and lower halves of the wells for the experiments with gelatin and PLL coatings. When we calibrate the solution of the Fisher-Kolmogorov model to match the experimental replicates in Section 3.3, we combine data from the two types of control assays which we refer to as the *regrouped control experiment*.

2.2 Edge detection and cell counting methods

We use ImageJ to detect edges of vacant areas and measure the gap areas from the experimental images at $t = 0, 12, 24, 36$, and 48 h (Schindelin et al., 2015; Simpson et al., 2013). Details of the methods are described in Jin et al. (2018b). Examples of experimental images, each of which has a length of 1960 μm and a width of 1288 μm, with detected gap edges superimposed, are shown in Figure 2. In the remainder of this work we refer to the area contained in the experimental image as the *experimental field-of-view*, since these images show relatively small subregions within the entire experimental well (Simpson et al., 2018). Estimates of the gap width at each time point is calculated by taking the gap area divided by the width of the experimental field-of-view. This calculation is performed at each time point for each experimental replicate. Here we use $W^{(r)}(t)$ to denote the experimental measurement of the gap width for replicate r at time t. We then average the data to give $\widetilde{W}(t) = (1/R)\sum_{r=1}^{R} W^{(r)}(t)$, where R is the number of replicates. We report the sample mean as well as the variability across the replicates by calculating and reporting the sample standard deviation.

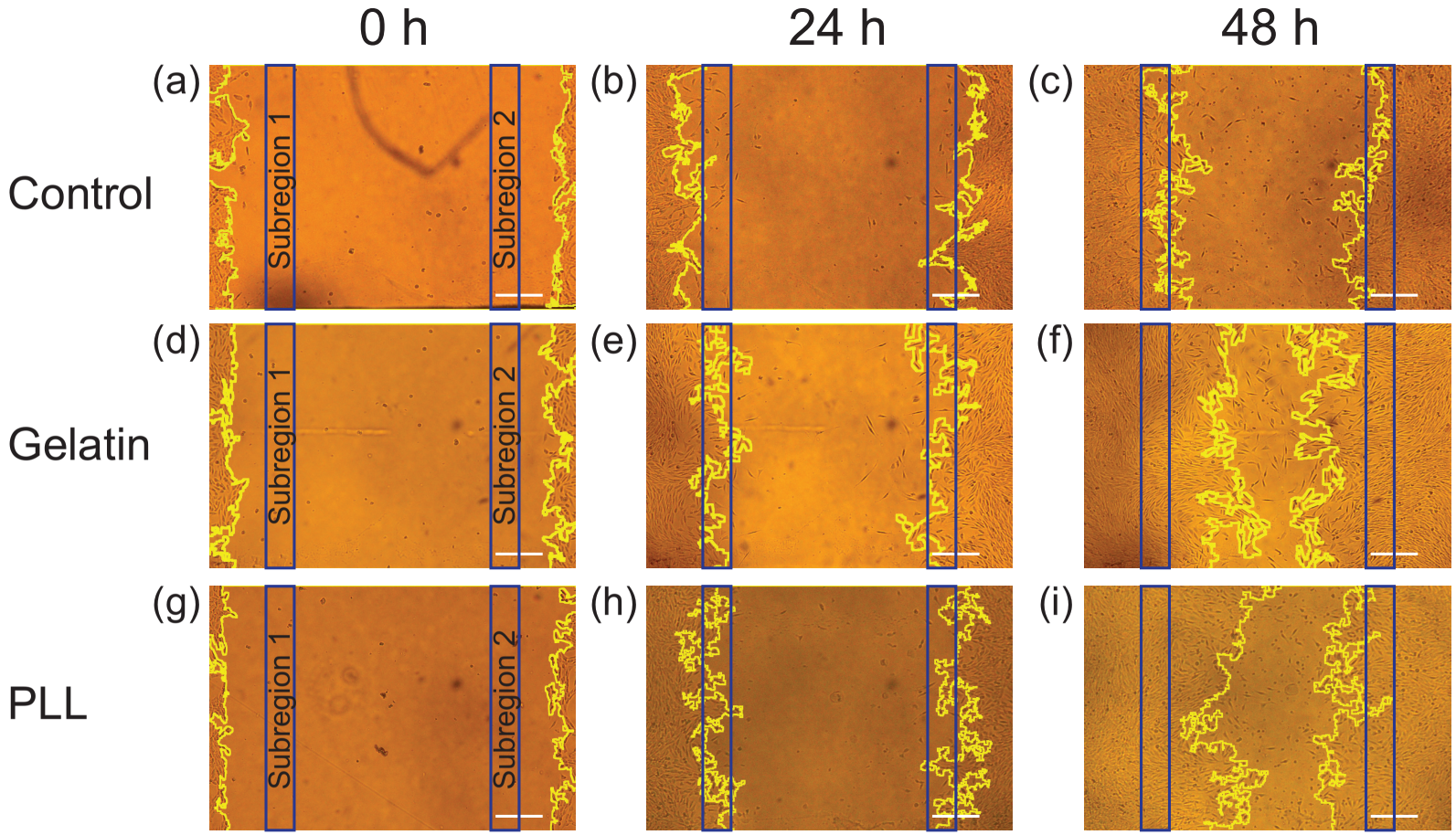

Fig. 2. **Experimental images at $t = 0$, 24, and 48 h from stopper assays.** (a)–(c) Experimental images of the control experiment. (d)–(f) Experimental images of the gelatin–coated experiments. (g)–(i) Experimental images of the PLL–coated experiments. All images show the experimental field-of-view in the upper half of the experimental well, indicated by the black arrow in Figure 1(f)–(g). The yellow line indicates the detected edge of the gap area produced by ImageJ. The rectangle highlighted in blue indicates the subregions in which cell numbers are counted over time. The scale bar corresponds to 500 μm.

We manually count the number of individual cells contained in two rectangular subregions at $t = 0, 12, 24, 36$, and 48 h. Each subregion, highlighted in blue in Figure 2, measures 1288 μm $\times$ 150 μm. The two subregions are located 300 μm away from the left and right boundaries of the experimental field-of-view, respectively. For simplicity we refer to the left-most subregion as *Subregion 1* and the right-most subregion as *Subregion 2*. These estimates are obtained at each time point for each experimental replicate. Here we use $N_1^{(r)}(t)$ and $N_2^{(r)}(t)$ to represent the experimental measurement of the number of cells within Subregion 1 and Subregion 2 for replicate r at time t, respectively. We then average the data to give $\widetilde{N}_1(t) = (1/R)\sum_{r=1}^{R} N_1^{(r)}(t)$, and $\widetilde{N}_2(t) = (1/R)\sum_{r=1}^{R} N_2^{(r)}(t)$.

Since the Subregion 1 and Subregion 2 are located symmetrically about the centre of the experimental field-of-view, we expect that the number of cells in each subregion will be approximately equal. Therefore, we average the number of cells in the two subregions for each experimental replicate to give $N^{(r)}(t) = \left(N_1^{(r)}(t) + N_2^{(r)}(t)\right)/2$. We also average this quantity across the experimental replicates to give $\widetilde{N}(t) = \left(\widetilde{N}_1(t) + \widetilde{N}_2(t)\right)/2$. Again, we report these averaged quantities and we approximate the uncertainty in these quantities by calculating and reporting the sample standard deviation.

2.3 Mathematical model: the Fisher–Kolmogorov model

The Fisher-Kolmogorov model is a reaction–diffusion equation given by

$$\frac{\partial \bar{C}(x,y,t)}{\partial t} = \overbrace{D\nabla^2 \bar{C}(x,y,t)}^{\text{linear diffusion}} + \overbrace{\lambda \bar{C}(x,y,t)\left(1 - \frac{\bar{C}(x,y,t)}{K}\right)}^{\text{logistic growth}}, \tag{1}$$

on $0 \leq x \leq X$, $0 \leq y \leq Y$, where X and Y are the horizontal length and vertical height of the experimental field-of-view, respectively (see Figure 3(a)–(b)), and $t \geq 0$ is time. In this model $\bar{C}(x,y,t) \geq 0$ [cells/μm^2] is the cell density, $D \geq 0$ [μm^2/h] is the cell diffusivity, $\lambda \geq 0$ [/h] is the cell proliferation rate, and $K > 0$ [cells/μm^2] is the carrying capacity density. Since all cells, both inside and outside of the experimental field-of-view, are uniformly seeded we define our simulation domain to be a horizontal extension of the experimental field-of-view, by doubling its length to avoid boundary effects. Therefore, we solve Equation (1) on $-X/2 \leq x \leq 3X/2$, and $0 \leq y \leq Y$. Zero net flux boundary conditions are applied along the four boundaries (Jin et al. 2016).

Previously, Simpson (2009) showed that depth-averaging can be used to simplify two-dimensional reaction-diffusion models into one-dimensional reaction-diffusion models by averaging $\bar{C}(x,y,t)$ in the vertical direction,

$$C(x,t) = \frac{1}{Y}\int_0^Y \bar{C}(x,y,t)\ \mathrm{d}y, \tag{2}$$

where $C(x,t)$ [cells/μm^2] is the one-dimensional vertically averaged cell density. To simplify Equation (1) we integrate both sides of the reaction–diffusion equation with respect to y, and then divide both sides by Y to obtain

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + \frac{D}{Y}\left(\left.\frac{\partial C}{\partial y}\right|_Y - \left.\frac{\partial C}{\partial y}\right|_0\right) + \lambda C\left(1 - \frac{C}{K}\right). \tag{3}$$

We note that the second and third terms on the right side of Equation (3) vanish since $\partial C/\partial y = 0$ along the boundaries where $y = 0$ and $y = Y$. Therefore, Equation (3) reduces to

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + \lambda C\left(1 - \frac{C}{K}\right). \tag{4}$$

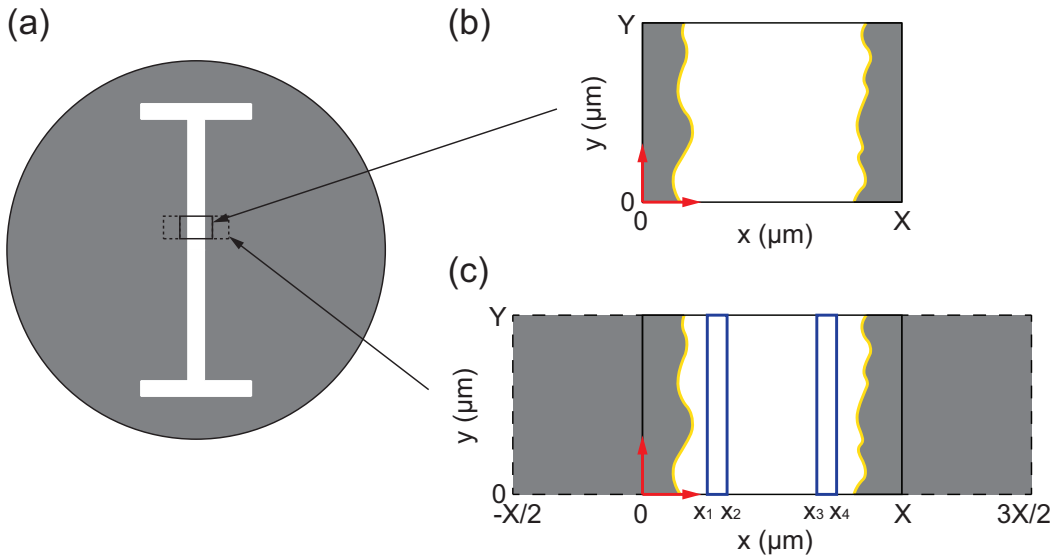

Fig. 3. **Schematics showing the entire experimental well, the experimental field-of-view, and the simulation domain.** (a) Entire experimental well. The white area shows the initial gap made by the stopper. The grey area shows the region where cells are uniformly seeded. (b) Experimental field-of-view, where $X = 1960$ μm, $Y = 1288$ μm and the origin and coordinate system is shown in red. The yellow curve highlights the gap edges. (c) Simulation domain. The inner rectangular region (solid lines) shows the actual experimental field-of-view. The outer rectangular region (dashed lines) shows the extended simulation domain. The blue rectangular subregions indicate the areas where cell numbers are counted.

It is worth noting that simplifying a two–dimensional reaction–diffusion model into a vertically averaged one–dimensional model can introduce an error. However, this error vanishes when the initial condition is uniform in the vertical direction (Simpson, 2009). Since the stopper assays do not involve any cell density gradients in the vertical direction, there is no error associated with averaging the two–dimensional reaction–diffusion equation into the simpler one–dimensional reaction–diffusion equation.

2.3.1 Estimating gap width using the Fisher-Kolmogorov model

One feature of the Fisher-Kolmogorov model is that its solutions do not have compact support (McCue et al., 2019). A level set $\epsilon \in [0, K]$, needs to be nominated to represent edges of the gap. The choice of ϵ also affects the computation of gap width, $\mathcal{W}(t)$. To estimate $\mathcal{W}(t)$ from the numerical solution of Equation (4), we calculate the x coordinates, x_{l} and x_{r}, at left and right side of the gap, respectively, which satisfies $C(x_{\mathrm{l}}, t) = C(x_{\mathrm{r}}, t) = \epsilon$. With this data the gap width is given by $\mathcal{W}(t) = x_{\mathrm{r}} - x_{\mathrm{l}}$, provided $x_{\mathrm{r}} > x_{\mathrm{l}}$. Otherwise $\mathcal{W}(t) = 0$. When we calibrate the mathematical model to a particular experimental replicate we use a superscript to denote the particular replicate. For example, $\mathcal{W}^{(r)}(t)$ denotes the gap width predicted by the model for experimental replicate r.

2.3.2 Estimating the number of cells using the Fisher-Kolmogorov model

The number of cells within Subregion 1 and Subregion 2 can be calculated by integrating the cell density, $C(x, t)$, over the two subregions, giving

$$\mathcal{N}_1(t) = Y \int_{x_1}^{x_2} C(x, t)\, \mathrm{d}x, \qquad \text{and} \qquad \mathcal{N}_2(t) = Y \int_{x_3}^{x_4} C(x, t)\, \mathrm{d}x, \tag{5}$$

where $C(x, t)$ is the cell density obtained by solving Equation (4) numerically. We further average $\mathcal{N}_1(t)$ and $\mathcal{N}_2(t)$ to give

$$\mathcal{N}(t) = \frac{\mathcal{N}_1(t) + \mathcal{N}_2(t)}{2}. \tag{6}$$

Again, when we calibrate the mathematical model to a particular experimental replicate we use a superscript to denote the particular replicate. For example, $\mathcal{N}^{(r)}(t)$ denotes the number of cells predicted by the model for experimental replicate r.

3 Results and discussion

3.1 Experimental estimates of $\widetilde{W}(t)$ and $\widetilde{N}(t)$

Using the methods described in Section 2.2, we obtain estimates of $\widetilde{W}(t)$ and $\widetilde{N}(t)$ for the gelatin, PLL, and control experiments (Figure 4(a)–(b)). This average data indicates that $\widetilde{W}(t)$ decreases fastest for the gelatin–coated experiments and slowest for the control experiments. We also see similar trends in terms of $\widetilde{N}(t)$ data. Starting with $\widetilde{N}(0) = 0$ in all cases, the experiments with gelatin coating lead to the fastest increase in $\widetilde{N}(t)$ while the control experiments lead to the slowest increase in $\widetilde{N}(t)$.

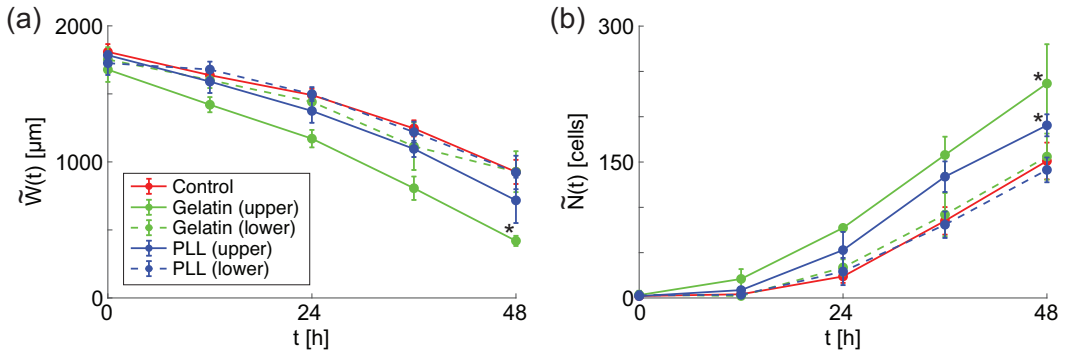

Fig. 4. **Time evolution of $\widetilde{W}(t)$ and $\widetilde{N}(t)$** (a) Time evolution of $\widetilde{W}(t)$ for the control, gelatin, and PLL experiments. (b) Time evolution of $\widetilde{N}(t)$ for the control, gelatin, and PLL experiments. Data points correspond to the sample mean and the error bars indicate the sample standard deviation. The asterisk indicates the data with which the null hypothesis is rejected at the 5% level.

To statistically identify the significance of difference in both the $\widetilde{W}(t)$ and $\widetilde{N}(t)$ data between the control and coating experiments, we conduct a two-sample t-test for $\widetilde{W}(48)$ and $\widetilde{N}(48)$, respectively, with the null hypothesis that the data in the control and coating experiments comes from independent random samples from normal distributions with equal means and equal but unknown variances (De Winter, 2013). The null hypothesis is rejected at the 5 % level ($p < 0.05$) for the experiments with gelatin coating in the $\widetilde{W}(48)$ data, and for

the experiments with gelatin and PLL coatings in the $\widetilde{N}(48)$ data. We obtain much larger p values when considering results for the lower half of the wells in the gelatin- and PLL-coated experiments, where the surface is uncoated. This result confirms our assumption that the two different types of control experiments we consider lead to indistinguishable results.

In summary, our experimental data shows that different coatings lead to different estimates of $\widetilde{W}(t)$ and $\widetilde{N}(t)$. For example, both coatings lead to $\widetilde{N}(t)$ that increases faster than $\widetilde{N}(t)$ for the control experiments. One naive way to interpret this difference is that the coatings stimulate cell proliferation. However, as we will show in Section 3.3, when we carefully interpret the experimental data using the Fisher–Kolmogorov model it suggests that the coatings stimulate cell migration but not cell proliferation.

To match the number of cells data with the solution of the Fisher-Kolmogorov model, we integrate both sides of Equation (4) with respect to x across the two subregions to give

$$\frac{\mathrm{d}\mathcal{N}_1}{\mathrm{d}t} = \overbrace{-D\frac{\partial C}{\partial x}\bigg|_{x_1}}^{\substack{\text{flux into the left boundary} \\ \text{of Subregion 1}}} + \overbrace{D\frac{\partial C}{\partial x}\bigg|_{x_2}}^{\substack{\text{flux out of the right boundary} \\ \text{of Subregion 1}}} + \overbrace{\lambda\int_{x_1}^{x_2} C\left(1-\frac{C}{K}\right)\mathrm{d}x}^{\substack{\text{population change in Subregion 1} \\ \text{due to cell proliferation}}}, \tag{7}$$

$$\frac{\mathrm{d}\mathcal{N}_2}{\mathrm{d}t} = \overbrace{-D\frac{\partial C}{\partial x}\bigg|_{x_3}}^{\substack{\text{flux into the left boundary} \\ \text{of Subregion 2}}} + \overbrace{D\frac{\partial C}{\partial x}\bigg|_{x_4}}^{\substack{\text{flux out of the right boundary} \\ \text{of Subregion 2}}} + \overbrace{\lambda\int_{x_3}^{x_4} C\left(1-\frac{C}{K}\right)\mathrm{d}x}^{\substack{\text{population change in Subregion 2} \\ \text{due to cell proliferation}}}. \tag{8}$$

Equations (7)–(8) show that the rate of change of cell numbers in particular subregions is driven by a combination of the net flux of cells across the boundaries of the subregion and the effect of proliferation within the subregion. Therefore, the increase in $\mathcal{N}(t)$ is driven by both the migration term and the proliferation term in Equation (4). Similarly the decrease in the width of the gap, $\mathcal{W}(t)$, is also driven by the combined migration and proliferation terms in Equation (4). Without carefully interpreting the experimental data

using a mathematical model it could be very difficult to separate the effects of cell migration from cell proliferation in these kinds of experiments. We will now calibrate the solution of Equation (4) to match the data for individual replicates, but first we will estimate the carrying capacity density and the initial condition separately.

3.2 Specifying the carrying capacity density and the initial condition

We directly measure the carrying capacity density, K, from the experimental images (Supplementary Material). Direct counting of maximum cell densities at the final time point of the experiments gives $K = 2.43 \pm 0.36 \times 10^{-3}$ cells/μm^2. We find that our estimates of K are very similar for the control, PLL- and gelatin-coated experiments so we have pooled all these estimates together (Supplementary Material). Since we find that K does not vary significantly between different replicates or between different coating conditions, we treat K as a constant.

Unlike the carrying capacity density, we find that there are some differences in the details of the initial conditions in the various experimental replicates. Therefore we specify a different initial condition for each replicate by counting cells away from the initial gap. The initial condition for each replicate is given by

$$C^{(r)}(x,0) = \begin{cases} \mathcal{C}_0^{(r)}, & x \leq \left(X - W^{(r)}(0)\right) 2, \\ 0 & \left(X - W^{(r)}(0)\right)/2 < x < \left(X + W^{(r)}(0)\right)/2, \\ \mathcal{C}_0^{(r)}, & x \geq \left(X + W^{(r)}(0)\right)/2, \end{cases} \tag{9}$$

where $W^{(r)}(0)$ is the initial gap width in experimental replicate r, and $\mathcal{C}_0^{(r)}$ is the cell density away from the initial gap in experimental replicate r. We extract these quantities from each experimental replicate and report them in the Supplementary Material document.

3.3 Calibrating the cell diffusivity and the cell proliferation rate

In this section we calibrate the solution of Equation (4) to match both the $W^{(r)}(t)$ and $N^{(r)}(t)$ data from each individual experimental replicate. We solve Equation (4) using a finite difference method, with our measurements of $C^{(r)}(x,0)$ and K (Supplementary Material). In Figure 5(a)–(c) we plot the solution of Equation (4), $C(x,t)$ at $t = 0, 12, 24, 36$, and 48 h, for replicate 2 of the regrouped control, and experiments with gelatin and PLL coatings, respectively. Using these solutions we compute $\mathcal{W}(t)$ and $\mathcal{N}(t)$, and with these estimates we calibrate D, λ and ϵ using MATLAB's *lsqcurvefit* algorithm (MathWorks, 2020) to provide the best match to the experimental data (Supplementary Material). We use a least–squares measure of the discrepancy between the data and the model solution, given by

$$E^{(r)}(D,\lambda,\epsilon) = \sum_{j=1}^{5}\left[\frac{\mathcal{W}(t_j) - W^{(r)}(t_j)}{W^{(r)}_{\max}}\right]^2 + \left[\frac{\mathcal{N}(t_j) - N^{(r)}(t_j)}{N^{(r)}_{\max}}\right]^2, \qquad (10)$$

where j is an index indicating the time points, and $W^{(r)}_{\max}$ and $N^{(r)}_{\max}$ are the largest gap width and number of cells in experimental replicate r, respectively. For the experiments with gelatin and PLL coatings, we have $r = 1, 2$, and 3, while for the regrouped control experiment, $r = 1, 2, \ldots, 9$. Equation (10) measures the difference between the model predictions and experimental data, both scaled by the maximum value in the experimental observations. This approach of scaling normalises the two different types of the data so that both of them are in the same order of magnitude. Calibrating the solution of the Fisher-Kolmogorov model to match the data allows us to estimate values of D, λ and ε that minimise $E^{(r)}$, and we denote these estimates using an overbar, $\bar{D}^{(r)}$, $\bar{\lambda}^{(r)}$ and $\bar{\epsilon}^{(r)}$ (Supplementary Material). Since these estimates do not vary too much across the different replicates, we average them across the replicates to give $\bar{D}$, $\bar{\lambda}$, $\bar{\epsilon}$, together with estimates of variability across the replicates in Table 1.

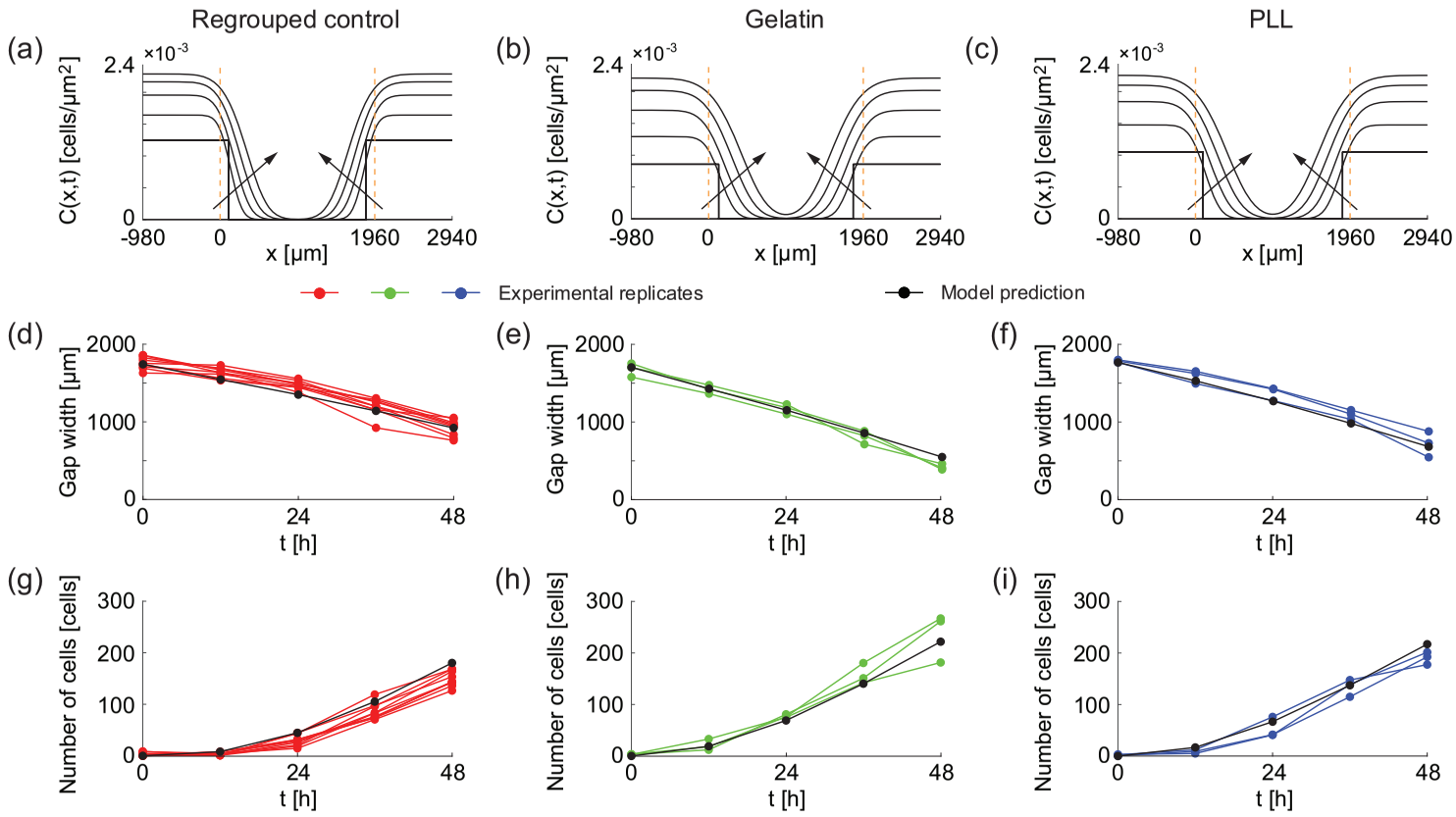

Fig. 5. **Comparing calibrated solutions of the Fisher-Kolmogrov model and the experimental data.** (a)–(c) Numerical solutions of Equation (4) at $t = 0, 12, 24, 36$, and 48 h, using $\bar{D}$ and $\bar{\lambda}$, with $C^{(2)}(x, 0)$ and $W^{(2)}(0)$ for experimental replicate 2. The arrow indicates the direction of increasing t. The orange dashed vertical lines indicate the experimental field-of-view. (d)–(f) Time evolution of $\mathcal{W}^{(2)}(t)$ from the calibrated solution of Equation (4) superimposed on the data, $W^{(r)}(t)$, $r = 1, \ldots, R$. (g)–(i) Time evolution of $\mathcal{N}(t)$ from the calibrated solution of Equation (4) superimposed on the data, $N^{(r)}(t)$, $r = 1, \ldots, R$. In (d), (g), $R = 9$. In (e), (f), (h), (i), $R = 3$.

Table 1

Estimates of $\bar{D}$, $\bar{\lambda}$, and $\bar{\epsilon}$ from individual experimental data. All parameter estimates are given to two significant figures.

	$\bar{D}$ (μm^2/h)	$\bar{\lambda}$ (/h)	$\bar{\epsilon}$ (% of K)
Control	600 ± 240	0.057 ± 0.0061	17 ± 2.7
Gelatin	1000 ± 140	0.061 ± 0.019	12 ± 2.9
PLL	1000 ± 390	0.059 ± 0.012	17 ± 2.9

Our estimates of $\bar{D}$, $\bar{\lambda}$ and $\bar{\epsilon}$ are within previously reported ranges for fibroblast cells (Jin et al., 2018b; Simpson et al., 2013; Tremel et al., 2009). Comparing estimates of $\bar{D}$ and $\bar{\lambda}$ indicates that both the gelatin and PLL coatings stimulate cell migration by about 70% compared to the regrouped control experiment. However, the proliferation rate $\bar{\lambda}$ does not vary over the three groups. Therefore, we find that gelatin and PLL coatings lead to increased cell motility, whereas neither coatings have any significant impact on cell proliferation.

Furthermore, our estimates of $\bar{D}$ for the gelatin and PLL coatings are very similar, suggesting that the impact of gelatin and PLL coatings on cell motility is similar. Results in Figure 5(d)–(i) show $\mathcal{W}^{(2)}(t)$ and $\mathcal{N}^{(2)}(t)$ obtained using the calibrated parameter values, superimposed with the $W^{(r)}(t)$ and $N^{(r)}(t)$ data for the individual experimental replicates. These results confirm that the calibrated solution of the Fisher–Kolmogorov model is consistent with the experimental data.

4 Conclusions

In vitro experimental approaches often study the effect of surface coatings simply by comparing images of experiments with coatings to control experiments without coatings. While such comparisons provide information about the net effect of the coating, the simple observations do not provide any insight into

how the coating affects the cell-level mechanisms. For example, observing that a particular coating increases the rate of gap closure provides no insight into whether the increase in the closure rate is driven by an increase in cell motility, an increase in cell proliferation, or a combined increase in cell motility and cell proliferation.

In this work we take a different, more quantitative approach to assess the impact of different coatings on *in vitro* wound–healing experiments. We are motivated to take this approach because we aim to understand how different coatings affect different mechanisms. We consider three types of experiments including control experiments without any coating as well as experiments with gelatin and PLL coatings. In each experiment we extract two types of data: (i) the gap width as a function of time; and (ii) the number of cells contained within particular subregions as a function of time. By carefully calibrating the solution of the Fisher–Kolmogorov model to match both types of data we obtain estimates of the cell diffusivity, D, and the cell proliferation rate, λ. Comparing estimates of D and λ between the control experiments and the experiments with gelatin and PLL coatings indicates that these two coatings increase cell migration by approximately 70% whereas the coatings have negligible impact on cell proliferation.

Overall, our results suggest that care ought to be taken when interpreting the experimental data. For example, simply counting the number of cells within particular subregions of the experiment shows that the number of cells increases more dramatically in the experiments with PLL and gelatin coatings. Our modelling suggests that this increase in cell number is driven by the coatings stimulating cell migration without influencing the rate of cell proliferation. This conclusion might not be obvious without interpreting our experimental data with a mechanistic mathematical model.

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Supplementary Material: Quantifying the role of different surface coatings in experimental models of wound healing

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Contents

1 Numerical methods for solving the Fisher-Kolmogorov model

The one-dimensional Fisher-Kolmogorov equation is of the form

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} + \lambda C\left(1 - \frac{C}{K}\right), \tag{S1}$$

on $-980 \leq x \leq 2940$ μm, where D [μm^2/h] is the cell diffusivity, λ [/h] is the cell proliferation rate, and K [cells/μm^2] is the carrying capacity density. To numerically solve Equation (S1), the spatial domain is discretised into M nodes using a central difference approximation with uniform spacing δx. Here we denote C_i as the cell density at a discretised node i, where $i = 1, 2, 3, \ldots, M$. The discretisation for the internal nodes at time t is as follows

$$\frac{\mathrm{d}C_i(t)}{\mathrm{d}t} = \frac{D}{\delta x^2}\Big(C_{i+1}(t) - 2C_i(t) + C_{i-1}(t)\Big) + \lambda C_i(t)\left(1 - \frac{C_i(t)}{K}\right), \tag{S2}$$

for $i = 2, \ldots, M-1$. We apply zero net flux boundary condition at both boundaries, i.e., $C_2 = C_1$ and $C_M = C_{M-1}$. The initial condition is obtained by measuring cell densities from the experimental images at $t = 0$ h, with the average data and data for individual replicates given in Table S5 and Table S16, respectively. The resulting system of nonlinear ordinary differential equations is integrated using a backward Euler method with constant time step δt, which leads to a system of coupled nonlinear algebraic equations linearised and solved using the Thomas algorithm, with absolute tolerance η (Morton and Mayers, 2005). For all results presented in the main manuscript as well as the supplementary material, we choose $\delta x = 0.5$ μm, $\delta t = 0.1$ h, and $\eta = 1 \times 10^{-5}$ so that our results are grid-independent.

2 Model calibration and additional parameter estimates data

We numerically solve the one-dimensional Fisher-Kolmogorov model (Equation (S1)) using a finite difference method, with $C^{(r)}(x,0)$ and K measured from the experimental images. Using the numerical methods introduced in Section 1, we obtain the density profiles at $t = 0, 12, 24, 36$, and 48 h. We then compute $\mathcal{W}(t)$ and $\mathcal{N}(t)$, and with these estimates we calibrate D and λ for the regrouped control, gelatin, and PLL experiments. We systematically vary ϵ, from $0.01K - 0.25K$, to identify the level set that minimises the least-squares measure of the discrepancy between the data and the model solution, given in Equation (10) in the main manuscript.

To consider the variation in the parameter estimates, we calibrate the solution of Equation (S1) to match the $W^{(r)}(t)$ and $N^{(r)}(t)$ data from each individual replicate. We find that each case appears to have a well-defined minimum, from which we estimate $\bar{D}^{(r)}$, $\bar{\lambda}^{(r)}$, and $\bar{\epsilon}^{(r)}$. We then average them across the replicates to give $\bar{D}$, $\bar{\lambda}$, and $\bar{\epsilon}$, which are listed in Table 1 in the main manuscript. In this supplementary material, we show histograms of the parameter estimates of the cell diffusivity $\bar{D}^{(r)}$ and proliferation rate $\bar{\lambda}^{(r)}$ for individual replicates in Figure S1. The estimated parameter values for individual replicates are listed in Table S1 - Table S3.

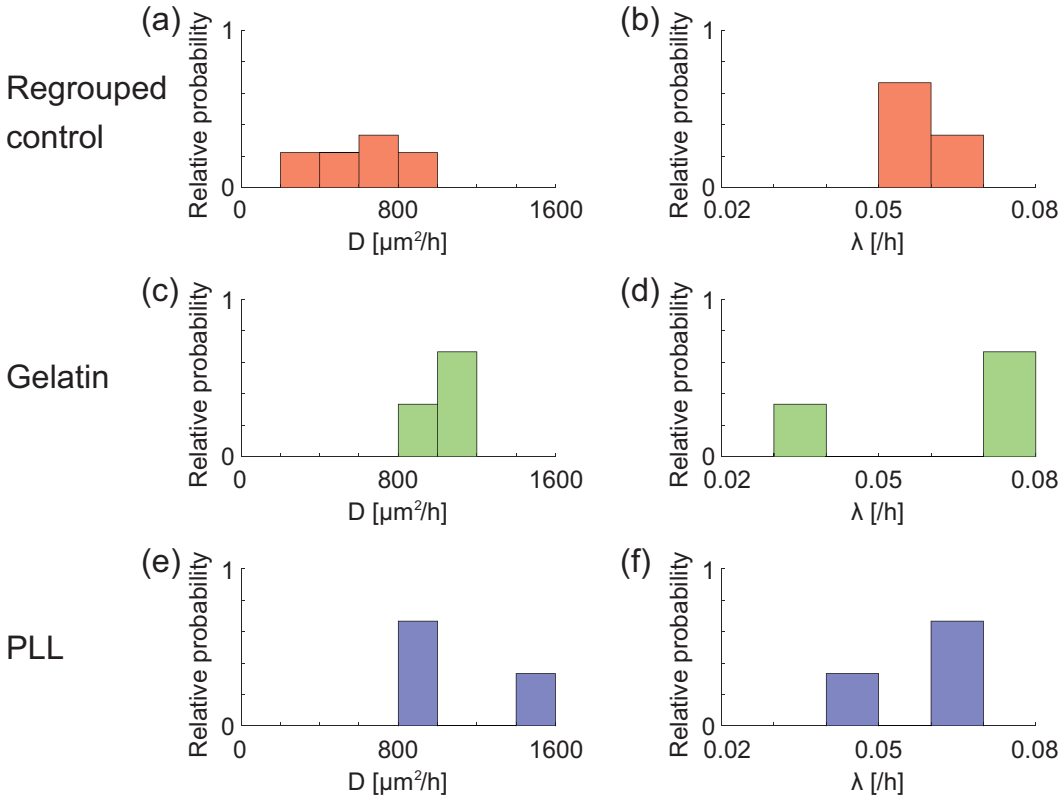

Fig. S1. **Histograms of parameter estimates of the cell diffusivity $\bar{D}^{(r)}$ and proliferation rate $\bar{\lambda}^{(r)}$.** The three rows correspond to the parameter estimates for the regrouped control, gelatin, and PLL experiments, respectively.

Table S1

Estimates of $\bar{D}$ and $\bar{\lambda}$ for individual replicates in the regrouped control experiment. All parameter estimates are given to two significant figures. The red column in each replicate indicates the level set which gives the minimum least-squares measure.

Regrouped control		Level set ϵ (% of K)					
		1	5	10	15	20	25
Replicate 1	$\bar{D}^{(1)}$ [μm^2/h]	220	370	500	610	660	660
	$\bar{\lambda}^{(1)}$ [/h]	0.12	0.088	0.075	0.068	0.066	0.066
	$E^{(1)}$ ($\times 10^{-2}$)	13	5.8	2.7	1.4	1.4	2.5
Replicate 2	$\bar{D}^{(2)}$ [μm^2/h]	180	290	380	460	490	500
	$\bar{\lambda}^{(2)}$ [/h]	0.099	0.072	0.060	0.054	0.051	0.051
	$E^{(2)}$ ($\times 10^{-2}$)	12	4.9	1.8	0.53	0.43	1.2
Replicate 3	$\bar{D}^{(3)}$ [μm^2/h]	260	440	590	720	760	710
	$\bar{\lambda}^{(3)}$ [/h]	0.11	0.080	0.068	0.061	0.060	0.063
	$E^{(3)}$ ($\times 10^{-2}$)	14	6.0	2.3	0.85	1.1	2.7
Replicate 4	$\bar{D}^{(4)}$ [μm^2/h]	210	350	490	620	680	660
	$\bar{\lambda}^{(4)}$ [/h]	0.13	0.094	0.079	0.070	0.067	0.069
	$E^{(4)}$ ($\times 10^{-2}$)	12	5.4	2.1	0.71	0.56	1.5
Replicate 5	$\bar{D}^{(5)}$ [μm^2/h]	160	250	320	370	390	380
	$\bar{\lambda}^{(5)}$ [/h]	0.087	0.066	0.056	0.051	0.049	0.051
	$E^{(5)}$ ($\times 10^{-2}$)	9.6	3.6	1.1	0.36	0.63	1.6
Replicate 6	$\bar{D}^{(6)}$ [μm^2/h]	280	470	660	800	910	890
	$\bar{\lambda}^{(6)}$ [/h]	0.086	0.066	0.057	0.053	0.051	0.053
	$E^{(6)}$ ($\times 10^{-2}$)	14	6.5	3.5	2.9	4.1	7.1
Replicate 7	$\bar{D}^{(7)}$ [μm^2/h]	160	280	360	430	450	460
	$\bar{\lambda}^{(7)}$ [/h]	0.11	0.077	0.066	0.060	0.058	0.058
	$E^{(7)}$ ($\times 10^{-2}$)	16	7.9	4.0	2.2	1.6	1.9
Replicate 8	$\bar{D}^{(8)}$ [μm^2/h]	110	160	190	210	200	210
	$\bar{\lambda}^{(8)}$ [/h]	0.092	0.070	0.061	0.057	0.059	0.059
	$E^{(8)}$ ($\times 10^{-2}$)	8.2	4.1	2.6	2.2	2.3	2.8
Replicate 9	$\bar{D}^{(9)}$ [μm^2/h]	300	500	770	1000	1200	1100
	$\bar{\lambda}^{(9)}$ [/h]	0.093	0.072	0.059	0.052	0.050	0.052
	$E^{(9)}$ ($\times 10^{-2}$)	18	8.4	3.4	1.1	1.2	3.7

Table S2

Estimates of $\bar{D}$ and $\bar{\lambda}$ for individual replicates in the gelatin experiment. All parameter estimates are given to two significant figures. The red column in each replicate indicates the level set which gives the minimum least-squares measure.

Gelatin		Level set ϵ (% of K)				
		1	5	10	15	20
Replicate 1	$\bar{D}^{(1)}$ [μm^2/h]	360	590	830	1100	1400
	$\bar{\lambda}^{(1)}$ [/h]	0.11	0.088	0.078	0.071	0.066
	$E^{(1)}$ ($\times 10^{-2}$)	6.8	2.4	0.89	0.62	1.3
Replicate 2	$\bar{D}^{(2)}$ [μm^2/h]	340	560	830	1100	1500
	$\bar{\lambda}^{(2)}$ [/h]	0.10	0.083	0.073	0.067	0.061
	$E^{(2)}$ ($\times 10^{-2}$)	6.0	2.4	1.3	1.3	2.4
Replicate 3	$\bar{D}^{(3)}$ [μm^2/h]	360	650	1000	1500	2400
	$\bar{\lambda}^{(3)}$ [/h]	0.059	0.046	0.039	0.034	0.031
	$E^{(3)}$ ($\times 10^{-2}$)	8.3	3.2	2.1	3.1	5.8

Table S3

Estimates of $\bar{D}$ and $\bar{\lambda}$ for individual replicates in the PLL experiment. All parameter estimates are given to two significant figures. The red column in each replicate indicates the level set which gives the minimum least-squares measure.

PLL		Level set ϵ (% of K)					
		1	5	10	15	20	25
Replicate 1	$\bar{D}^{(1)}$ [μm^2/h]	240	380	520	660	800	880
	$\bar{\lambda}^{(1)}$ [/h]	0.12	0.093	0.080	0.072	0.066	0.064
	$E^{(1)}$ ($\times 10^{-2}$)	18	9.2	4.8	2.5	1.3	1.3
Replicate 2	$\bar{D}^{(2)}$ [μm^2/h]	450	760	1100	1500	2000	2200
	$\bar{\lambda}^{(2)}$ [/h]	0.078	0.061	0.052	0.046	0.042	0.043
	$E^{(2)}$ ($\times 10^{-2}$)	22	11	5.2	2.9	3.1	6
Replicate 3	$\bar{D}^{(3)}$ [μm^2/h]	250	440	630	840	1000	1100
	$\bar{\lambda}^{(3)}$ [/h]	0.11	0.086	0.074	0.066	0.061	0.062
	$E^{(3)}$ ($\times 10^{-2}$)	12	4.9	1.8	0.43	0.66	2.6

3 Experimental data describing the carrying capacity density, the initial cell density, and the initial gap width

In this section, we discuss the measure of carrying capacity density, K, and initial cell density, $\mathcal{C}_0^{(r)}$, for the experimental replicates. Our experimental results suggest that regions far behind the edges of the gap are fully occupied by cells after 48 h. Therefore, we directly count the number of cells at $t = 48$ h in the four identical 300 μm $\times$ 150 μm rectangular boxes, located 50 μm away from the edges of the experimental field-of-view (Figure S2). By averaging the number of cells over the four boxes in each replicate and then further averaging across the individual replicates, we obtain estimates of K listed in Table S4.

To estimate $\mathcal{C}_0^{(r)}$ we count and average the number of cells in the same four identically-sized rectangular boxes for replicate r at $t = 0$ h. In Table S5 we show the estimates of $\widetilde{\mathcal{C}}_0$ for the control, gelatin, and PLL experiments, obtained by averaging $\mathcal{C}_0^{(r)}$ across the individual replicates in each experiment.

In addition, in Table S6 we show the data of the average initial gap width, $\widetilde{W}(0)$, estimated using the edge detection method (Jin et al. 2018).

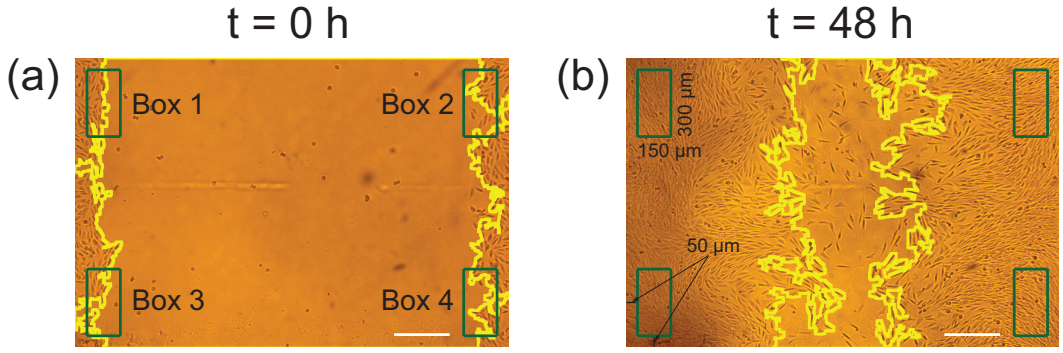

Fig. S2. **Examples of experimental images at $t = 0$ and 48 h.** The green rectangular boxes indicate regions where cell number is counted. The yellow lines indicate the gap edges. The scale bar corresponds to 500 μm.

Table S4

Estimates of K obtained by measuring and averaging cell numbers in the four identically-sized boxes in experimental images at $t = 48$ h. All estimates are rounded to two decimal places.

K [cells/μm^2]		Average from replicates	Standard deviation
Control		2.46×10^{-3}	3.88×10^{-4}
Gelatin	Upper	2.48×10^{-3}	8.94×10^{-5}
	Lower	2.34×10^{-3}	6.58×10^{-5}
PLL	Upper	2.41×10^{-3}	8.14×10^{-5}
	Lower	2.45×10^{-3}	7.59×10^{-5}
Average from experiments		2.43×10^{-3}	3.60×10^{-4}

Table S5

Estimates of $\widetilde{\mathcal{C}}_0$ obtained by measuring and averaging cell numbers in the four identically-sized boxes in experimental images at $t = 0$ h. All estimates are rounded to two decimal places.

$\widetilde{\mathcal{C}}_0$ [cells/μm^2]		Average from replicates	Standard deviation
Control		1.00×10^{-3}	4.15×10^{-4}
Gelatin	Upper	9.81×10^{-4}	3.51×10^{-4}
	Lower	1.03×10^{-3}	2.99×10^{-4}
PLL	Upper	9.39×10^{-4}	3.02×10^{-4}
	Lower	1.18×10^{-3}	5.35×10^{-4}
Average from experiments		1.02×10^{-3}	3.91×10^{-4}

Table S6

Estimates of $\widetilde{W}(0)$ measured from experimental images at $t = 0$ h. All estimates are rounded to the nearest integer.

$\widetilde{W}(0)$ [μm]		Average from replicates	Standard deviation
Control		1809	57
Gelatin	Upper	1678	90
	Lower	1753	90
PLL	Upper	1785	17
	Lower	1726	87
Average from experiments		1750	51

4 Experimental data for individual replicates

Table S7 - Table S9 list the data of $W^{(r)}(t)$ and $N^{(r)}(t)$ for the three replicates in the control experiment. Table S10 - Table S12 list the data of $W^{(r)}(t)$ and $N^{(r)}(t)$ for the three replicates in the gelatin experiment. Table S13 - Table S15 list the data of $W^{(r)}(t)$ and $N^{(r)}(t)$ for the three replicates in the PLL experiment. Table S16 shows the data of $\mathcal{C}_0^{(r)}$ and $K^{(r)}$ measured from individual replicates.

Table S7

Experimental data of $W^{(1)}(t)$ and $N^{(1)}(t)$ for replicate 1 in the control experiment.

Control 1	$W^{(1)}(t)$ [μm]		$N^{(1)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(1)}(t)$	$N_2^{(1)}(t)$	$N_1^{(1)}(t)$	$N_2^{(1)}(t)$
0	1838	1816	5	3	5	4
12	1686	1680	2	1	3	7
24	1567	1498	12	24	15	33
36	1201	1195	104	62	103	116
48	830	841	164	155	217	137

Table S8

Experimental data of $W^{(2)}(t)$ and $N^{(2)}(t)$ for replicate 2 in the control experiment.

Control 2	$W^{(2)}(t)$ [μm]		$N^{(2)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(2)}(t)$	$N_2^{(2)}(t)$	$N_1^{(2)}(t)$	$N_2^{(2)}(t)$
0	1745	1737	0	0	2	3
12	1589	1534	9	7	7	9
24	1467	1427	23	35	21	47
36	1279	1290	76	63	76	89
48	996	983	113	153	120	185

Table S9

Experimental data of $W^{(3)}(t)$ and $N^{(3)}(t)$ for replicate 3 in the control experiment.

Control 3	$W^{(3)}(t)$ [μm]		$N^{(3)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(3)}(t)$	$N_2^{(3)}(t)$	$N_1^{(3)}(t)$	$N_2^{(3)}(t)$
0	1882	1835	4	0	0	0
12	1695	1639	0	1	3	0
24	1504	1485	20	9	14	32
36	1325	1186	22	120	129	61
48	1036	873	132	112	178	148

Table S10

Experimental data of $W^{(1)}(t)$ and $N^{(1)}(t)$ for replicate 1 in the gelatin experiment.

Gelatin 1	$W^{(1)}(t)$ [μm]		$N^{(1)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(1)}(t)$	$N_2^{(1)}(t)$	$N_1^{(1)}(t)$	$N_2^{(1)}(t)$
0	1753	1856	6	1	13	4
12	1421	1632	23	13	2	0
24	1185	1496	80	72	30	22
36	883	1264	179	182	73	94
48	388	971	270	264	181	147

Table S11

Experimental data of $W^{(2)}(t)$ and $N^{(2)}(t)$ for replicate 2 in the gelatin experiment.

Gelatin 2	$W^{(2)}(t)$ [μm]		$N^{(2)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(2)}(t)$	$N_2^{(2)}(t)$	$N_1^{(2)}(t)$	$N_2^{(2)}(t)$
0	1704	1711	6	0	1	3
12	1476	1639	10	14	0	3
24	1227	1383	103	59	52	35
36	713	923	151	151	80	158
48	461	761	255	268	156	180

Table S12

Experimental data of $W^{(3)}(t)$ and $N^{(3)}(t)$ for replicate 3 in the gelatin experiment.

Gelatin 3	$W^{(3)}(t)$ [μm]		$N^{(3)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(3)}(t)$	$N_2^{(3)}(t)$	$N_1^{(3)}(t)$	$N_2^{(3)}(t)$
0	1578	1691	4	2	0	0
12	1365	1534	38	28	1	7
24	1101	1444	68	82	13	50
36	823	1157	151	133	53	94
48	409	1052	174	189	160	112

Table S13

Experimental data of $W^{(1)}(t)$ and $N^{(1)}(t)$ for replicate 1 in the PLL experiment.

PLL 1	$W^{(1)}(t)$ [μm]		$N^{(1)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(1)}(t)$	$N_2^{(1)}(t)$	$N_1^{(1)}(t)$	$N_2^{(1)}(t)$
0	1800	1753	6	0	9	7
12	1653	1729	7	3	9	0
24	1429	1556	52	30	9	20
36	1153	1304	113	165	57	84
48	879	1035	190	213	163	90

Table S14

Experimental data of $W^{(2)}(t)$ and $N^{(2)}(t)$ for replicate 2 in the PLL experiment.

PLL 2	$W^{(2)}(t)$ [μm]		$N^{(2)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(2)}(t)$	$N_2^{(2)}(t)$	$N_1^{(2)}(t)$	$N_2^{(2)}(t)$
0	1767	1629	3	2	0	0
12	1495	1615	9	15	7	3
24	1274	1461	91	61	32	25
36	1034	1200	155	140	98	51
48	546	941	198	157	153	135

Table S15

Experimental data of $W^{(3)}(t)$ and $N^{(3)}(t)$ for replicate 3 in the PLL experiment.

PLL 3	$W^{(3)}(t)$ [μm]		$N^{(3)}(t)$ [cells]			
t [h]	Upper	Lower	Upper		Lower	
			$N_1^{(3)}(t)$	$N_2^{(3)}(t)$	$N_1^{(3)}(t)$	$N_2^{(3)}(t)$
0	1786	1796	0	1	0	1
12	1624	1693	17	1	1	1
24	1421	1478	42	41	28	61
36	1101	1153	129	101	113	82
48	727	791	150	235	154	153

Table S16

Experimental data of $\mathcal{C}_0^{(r)}$ and K for individual replicates in the three groups. All the data are given to two decimal places.

t [h]	$\mathcal{C}_0^{(r)}$ [$\times 10^{-3}$ cells/μm^2]								$K^{(r)}$ [$\times 10^{-3}$ cells/μm^2]							
	Upper				Lower				Upper				Lower			
	Box 1	Box 2	Box 3	Box 4	Box 1	Box 2	Box 3	Box 4	Box 1	Box 2	Box 3	Box 4	Box 1	Box 2	Box 3	Box 4
Control 1	1.47	1.10	0.73	0.30	0.97	1.10	0.90	0.93	2.09	2.18	2.31	2.38	1.98	1.60	3.00	2.73
Control 2	1.50	1.03	1.70	1.10	1.53	0.43	1.23	1.43	2.40	2.04	2.20	2.18	2.38	2.47	2.33	2.29
Control 3	0.43	1.57	0.40	1.20	1.10	0.83	0.73	0.37	3.51	2.16	2.44	2.53	2.89	2.73	2.76	2.40
Gelatin 1	1.47	0.83	0.77	0.97	0.73	0.70	1.00	1.03	2.76	2.58	2.53	2.76	2.58	2.60	2.38	2.31
Gelatin 2	1.33	0.83	0.63	0.63	0.87	1.13	0.80	0.80	3.18	2.38	2.96	3.04	2.00	2.31	2.20	2.60
Gelatin 3	1.70	1.10	0.87	0.63	1.47	1.70	1.10	1.07	1.82	1.64	2.47	1.64	2.42	2.09	2.60	2.04
PLL 1	1.10	0.93	1.47	0.60	0.80	1.03	1.17	0.63	2.38	2.27	2.49	2.36	2.62	2.44	2.71	1.84
PLL 2	1.43	0.83	1.07	0.87	2.07	1.87	1.93	1.47	2.07	2.07	2.84	1.98	2.09	2.07	2.16	2.11
PLL 3	1.03	0.60	0.53	0.80	1.07	0.80	0.73	0.53	2.60	2.82	1.89	3.20	2.80	2.71	2.42	1.84

References

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[2] Morton KW, Mayers DF, 2005. Numerical Solution of Partial Differential Equations. Cambridge University Press, Cambridge.