Using a continuum model to decipher the mechanics of embryonic tissue spreading from time-lapse image sequences: An approximate Bayesian computation approach

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Abstract

Advanced imaging techniques generate large datasets that are capable of describing the structure and kinematics of tissue spreading in embryonic development, wound healing, and the progression of many diseases. Information in these datasets can be integrated with mathematical models to infer important biomechanical properties of the system. Standard computational tools for estimating relevant parameters rely on methods such as gradient descent and typically identify a single set of optimal parameters for a single experiment. These methods offer little information on the robustness of the fit and are ill-suited for statistical tests of different experimental groups. To overcome this limitation and use large datasets in a rigorous experimental design, we sought an automated methodology that could integrate kinematic data with a mathematical model. Estimated model parameters are represented as a probability density distribution, which can be achieved by implementing the approximate Bayesian computation rejection algorithm. Here, we demonstrate this method with a 2D Eulerian continuum mechanical model of spreading embryonic tissue. The model is tightly integrated with quantitative image analysis of different sized embryonic tissue explants spreading on extracellular matrix (ECM). Tissue spreading is regulated by a small set of parameters including forces on the free edge, tissue stiffness, strength of cell-ECM adhesions, and active cell shape changes. From thousands of simulations of each experiment, we find statistically significant trends in key parameters that vary with initial size of the explant, e.g., cell-ECM adhesion forces are weaker and free edge forces are stronger for larger explants. Furthermore, we demonstrate that estimated parameters for one explant can be used to predict the behavior of other explants of similar size. The predictive methods described here can be used to guide further experiments to better understand how collective cell migration is regulated during development and dysregulated during the metastasis of cancer.

Author Summary

New imaging tools and automated microscopes are able to produce terabytes of detailed images of protein activity and cell movements as tissues change shape and grow. Efforts to infer useful quantitative information from these large datasets rely on careful experimental design that incorporates image analysis, computational models, and rigorous statistical methods. In this paper, we describe a robust methodology for inferring mechanical processes that drive tissue spreading in embryonic development. Tissue spreading is critical during wound healing and the progression of many diseases including cancer. Direct measurement of biomechanical properties is not possible in many cases, but can be inferred through mathematical and statistical means. We developed a methodology to estimate mathematical model parameters to fit computer simulations with experimental videos of spreading embryonic tissue explants from the aquatic frog *Xenopus laevis*. Model parameters inferred with this approach are able to reliably predict results of new experiments. These methods can be applied to guide further experiments to better understand how tissue spreading is regulated during development and potentially control spreading during wound healing and cancer.

Introduction

A fundamental understanding of the physical mechanisms driving morphogenesis during embryogenesis, during disease progression, and within biosynthetic engineered structures is progressing rapidly through a powerful combination of advanced microscopy tools and detailed biophysical models. With advances in microscopy, very large time-lapse datasets can be generated over the course of a few hours. Such time-lapse data contains a wealth of information describing both the structures involved in morphogenesis and their kinematics; this information can serve as the input for computational models that allow us to explore the biological and biophysical principles of morphogenesis and predict the behavior of cells and tissues under a variety of perturbations. This presents many technical challenges, from collecting quality images suitable for machine vision tools to

developing computational models that implement appropriate biophysics and physiological rules at useful spatial and temporal scales. To maximize the benefits of these approaches, experimental designs must be able to integrate image data with model simulations in a way that allows robust statistical assessment of model fitness or failure.

Various theoretical and computational frameworks have been used to study tissue spreading and collective cell migration in cases such as wound healing and angiogenesis, and have included reaction-diffusion equations [1–10]; continuum mechanical models [11–17]; agent-based models [18–22]; vertex models [23–27]; finite element method (FEM) and CellFIT based inference methods [28,29]; and Vicsek and active elastic sheet models [30,31]. These models differ in their assumptions regarding, for example, whether individual cells are distinguishable within the tissue, the basic mechanical nature of the tissue, if cell signaling or mechanical forces are the primary drivers behind cell migration, and whether they assume a frame of reference that travels with the cells, i.e. a Lagrangian frame of reference, or a fixed frame that cells move across, i.e. an Eulerian frame of reference.

We seek to design studies that integrate time-lapse image data with computational models in order to reveal differences in mechanical processes as experimental conditions are varied. A standard method of estimating physically relevant model parameters is through deterministic approaches such as the gradient descent method. Deterministic approaches identify a single set of optimal model parameters that offer a best fit to experimental data [32]. However, deterministic approaches do not quantify uncertainty in either the source data or the mathematical model, and thus are ill-suited for testing the significance of parameter fits between two experimental groups. Thus, we implement a Bayesian method that attempts to define a probability density distribution for the estimated model parameters. In particular, we implement the approximate Bayesian computation (ABC) rejection algorithm [33,34] to estimate biomechanical properties of spreading embryonic tissue via a mathematical model of collective cell migration. ABC has also been used in studying complex

biological problems such as in population genetics, ecology, epidemiology, systems biology, and cell biology [33–36].

While our main goal is to develop a methodology for combining image data and computational modeling, here we apply this methodology to study the collective migration of tissue explants of various initial sizes. Epiboly is a key tissue movement during gastrulation in *Xenopus laevis* embryos, and it describes collective cell movements of the ectoderm of the animal cap region as is spreads and maintains coverage over the exterior of the embryo [37]. We make use of an *in vitro* model of epiboly where ectoderm from the late blastula stage animal cap region of the *Xenopus* embryo is microsurgically dissected and allowed to adhere to a fibronectin coated substrate. Dissected tissues, e.g. explants, spread over 10 to 24 hours in a manner similar to that observed of the tissue *in vivo*, suggesting that this spreading is at least partially driven by active processes in the ectoderm. Deep cells throughout the ectoderm are thought to contribute to epiboly through adhesion to and traction on a fibronectin coated substrate [38]. Cells at the margin of the explant are thought to generate outwardly directed traction forces that aid spreading. Additionally, as the tissue spreads, the cells of the ectoderm decrease in height but it is not known if this activity is active or merely due to passive strain in the tissue. Our goal in integrating time-lapse image data with a computational model is to determine the extent to which cell shape change, traction forces, adhesion, and tissue elasticity contribute to the mechanics of tissue spreading.

The physical mechanisms of *Xenopus* embryonic tissue spreading are investigated here by tracking both the movement of the tissue edge and internal tissue deformations. When cell nuclei can be tracked, internal deformations of spreading tissues may be tracked as local tissue density by counting cells in a subregion and then dividing by the area of that subregion [39–41]. Since tissues are generally opaque in the *Xenopus* ectoderm, we instead adopted an alternative automated method of extracting the local change in texture density between a pair of images in time-lapse sequences. This method involves calculating the strain, or local deformation, between a pair of images using elastic

registration [42]. Elastic registration, a form of differential image correlation [43], fits well with an elastic continuum model of tissue spreading, and it can also serve as a reasonable approximation for more general models of cell migration, such as ones that incorporate constitutive properties such as viscosity or plasticity [44].

In this paper, we adapt an established continuum mechanical mathematical model from Arciero et al. [11] for collective cell migration in which the model parameters correspond to physical quantities. We identify differences in physically meaningful parameters that regulate tissue spreading rates and deformations that depend on the initial explant size. Thousands of simulations of each experiment reveals multiple parameter regimes that lead to similar results. Single parameter sets identified using traditional deterministic methods may or may not represent these broader regimes and would be ill-suited for statistical tests. To identify parameters that modulate the role of initial explant size on spreading, we use approximate Bayesian computation (ABC) to determine the probability density distribution for each parameter and identify statistically significant differences using Tukey's multiple comparisons test.

Results

In order to explore the mechanical processes that are involved in the collective migration of developmental tissues, we microsurgical isolated ectodermal tissue from the animal cap region of gastrulating *Xenopus* embryos. During gastrulation, this tissue spreads to cover the exterior of the embryo (Fig 1A). Once isolated and placed on a fibronectin-coated substrate, tissue explants spread essentially symmetrically outward (Fig 1B). Fibronectin is the principle extracellular matrix ligand for animal cap cells and the number of binding sites is likely to control cell-substrate adhesion. The initial area of the explant affects the rate at which it spreads with larger explants spreading faster than smaller ones (Fig 1C-D), suggesting that the initial area of an explant affects the mechanics of the tissue. Since spreading rates may be affected by either the strength of adhesion, lamellipodia formation, or tissue

resistance to spreading from either its elastic modulus or the amount of material available, we use parameter estimation and statistical techniques to analyze predictions from simulations of a mathematical model to examine trends in estimated parameter values as they relate to the explants' initial areas. Experimental data such as tissue boundaries and strain are extracted from time-lapse sequences of images to integrate with the mathematical model (Fig 1E; Materials and Methods).

Fig 1. Schematics of experimental setup and numerical simulations.
(A) Schematic of animal cap ectoderm of a *Xenopus laevis* embryo during the late blastula/mid-gastrula stage and at the tailbud stage. At the late blastula stage, a single layer of epithelial cells (blue) lay on top of a deep multilayer of mesenchymal cells (red), which is on top of the blastocoel, a fluid filled cavity in the upper half of the embryo. The red bars indicate where an animal cap explant is cut from the embryo. By the tailbud stage, the ectoderm spreads to cover the entire embryo. The underlying mesenchymal multilayer has undergone intercalation to become a single layer while the cells in the epithelial layer stretch.
(B) Schematic of animal cap ectoderm removed from the embryo during the late blastula through mid-gastrula stages and cultured on fibronectin adsorbed glass initially (green), and then after time has passed. As the tissue spreads, deep mesenchymal cells radially intercalate into the epithelial cell layer (purple). Dashed box region shown enlarged below. Note: Experimental images are taken from above, not from the side, so only the pigment-containing apical surface of the epithelial layer is visible.
(C) Area of explants over course of the experiment for representative explants in the model building set (blue) and test set (red). Explants shown here are the same explants analyzed in Figure 4.
(D) Average change in area over time ($\Delta A/\Delta t$), calculated as the slope of the regression line of area measurements over time (as in panel C), for *Xenopus* animal cap explants of different initial sizes. The explants are grouped by initial area into subgroups Ia, Ib, II, and III, indicated by the dashed lines. The blue filled-in circles correspond with the explants included in our model building set and the red circles correspond with the explants included in our test set. The length of time was 10 hours.
(E) Time-lapse images of explant spreading are shown in the first row, followed by the boundary identification results of the Level Sets plugin. Then the strains as defined in Eq. 21 are shown and the density ratios (Eq. 16) are shown in the last row. See the Supporting Information Videos for time-lapse sequences of these still frames.

Our experimental data set contained 41 explants from two clutches of eggs. These explants were microsurgically shaped so the initial areas varied eighteen-fold (Fig 1C). We grouped these explants into four subgroups based on their initial area, where groups Ia and Ib are comprised of the smallest explants, group II has medium sized explants, and group III has large sized explants. We chose eighteen representative explants for our model building set to run the parameter estimation analysis on,

leaving the remaining explants available to test the predictive power of the method. See Fig 1C and Supporting Information S1 Table for the naming convention used for the explants and how it corresponds to the initial area of the explant (the smallest explant is named Explant #1).

Mathematical Model

To study *Xenopus* ectoderm explant spreading, we modify the two-dimensional (2D) cell migration model of Arciero et al. [11], which is derived from mechanics principles and uses a small number of parameters to capture many of the mechanisms that are important in 2D tissue spreading, in particular, adhesion between the tissue and substrate, elastic coupling of cells within the tissue, and forces generated by lamellipodia. This model also reflects the limited complexity of *Xenopus* explant mechanics and movements that we are able to capture through image analysis.

The model of Arciero et al. [11] was originally applied to small intestinal epithelial cell layers (IEC-6 cells) and Madin-Darby canine kidney (MDCK) epithelial cell layers. Minor modifications to the model are required to apply to explant spreading since the biological mechanisms of material growth in the visible cell layer are different. For example, epithelial cell layers in MDCK sheets are one cell thick and exhibit 2D volumetric growth through cell proliferation. By contrast, the *Xenopus* animal cap does not undergo 3D volumetric grow through cell proliferation [45] and is a composite tissue consisting of a single epithelial layer underlain by two or more layers of deep mesenchymal cells. Spreading in the animal cap can undergo apparent 2D volumetric growth as cells move from one layer to another. 2D volume (e.g. area) can change as superficial cells actively change shape or as deep cells move into the top layer from deeper within the tissue through radial intercalation (Fig 1B). (Note: *Xenopus* embryos do not change total volume from fertilization to feeding tadpole stages.) Since the time-lapse images of explant migration are taken normal to their plane of movement, we assume the explant tissue is a single layer that can be tracked using only the cells in the visible epithelial layer of

the tissue. Like the model for MDCK movement we also assume that the explant is uniformly thick, but note that changing this condition would be a useful future extension.

In the model of Arciero et al. [11], a 2D single cell layer is represented by an elastic continuum capable of deformation, motion, and proliferation of cells within the layer. Since the multicellular ectoderm of *Xenopus laevis* animal caps is continuous and does not break or shear, a continuum model of tissue migration is appropriate [46]. The tissue is represented as a 2D compressible inviscid fluid and the motion of cells is described in spatial (Eulerian) coordinates. The parameters in this model correspond to physical properties in the tissue, and thus we can deduce the material properties of the tissue by estimating model parameter values that best represent the behavior of an experiment. The governing equation of the model of Arciero et al. [11] is

$$\frac{\partial \rho}{\partial t} = \frac{k}{b}\Delta\rho + q, \qquad \text{in } \Omega^t, \tag{1}$$

where variable $\rho(\mathbf{x},t)$ describes the tissue density as a function of position $\mathbf{x}=(x,y)$ and time t, k and b are parameters described in Table 1, q is a growth term, and Ω^t is the domain that describes the extent of the tissue at time t (Fig 2A). See the Supporting Information S2 File or Arciero et al. [11] for further details on the derivation of Eq. 1 and the boundary conditions below.

Table 1. Mathematical model parameters, units, and descriptions.

Parameter	Units	Physical Description
k	force/length	Residual stretching modulus (stiffness) of the tissue. , Active rearrangements of the cytoskeleton are instantaneous relative to the timescale we use for comparing frames, therefore they are part of this residual stretching modulus.
b	force/area × (length/time)$^{-1}$	Adhesion constant between the mesenchymal cell layer and the fibronectin substrate.
F	force/length	Net external force that develops as a result of lamellipodia formation in the mesenchymal layer of the tissue. In the model, this is concentrated at the very edge of the tissue, but lamellipodia form across the basal surface of the mesenchyme.
$\rho_{\text{unstressed}}$	cells/length^2	Constant density of a relaxed (unstressed) tissue that occurs at equilibrium (i.e. if F=0 and the cell layer is not migrating).
α	time^{-1}	Visible material growth rate due to radial intercalation or active cell

		shape changes in the epithelial layer.

Fig 2. Schematics of the numerical simulations and approximate Bayesian computation rejection method.
(A) Computational domain of the moving boundary initial value problem as if looking at the animal cap explant from above. The tissue is located in the shaded region (Ω^t), the tissue boundary is denoted by $\partial\Omega_1^t$, the edge of the computational domain is denoted by $\partial\Omega_2$, and F is the net external force per unit length at the tissue boundary that develops as a result of lamellipodia formation.
(B) Schematic of a side-view look at the explant migration and the forces governing its movement. The green arrow represents the lamellipodia force F, the purple arrows represent the adhesion force b, and the spring represents the residual stretching modulus of the tissue k.
(C) Toy simulation showing the output of the boundary locations (as viewed from above) and density profiles (as a cross-section through the horizontal axis) every 2 hours from 0 to 10 hours. The fixed grid used is shown as gray dashed lines.
(D) Flow chart of the approximate Bayesian computation (ABC) rejection algorithm implemented for parameter estimation.

Given the presence of lamellipodia on the edge of the ectoderm, i.e. tissue boundary $\partial\Omega_1^t$, we assume there is a constant force per unit length F (see Table 1 and Fig 2A-B) exerted outward at the tissue boundary that is equal in magnitude to that of the force of the cells in the interior. To express this boundary condition in mathematical notation, a function describing the forces within the tissue is necessary. The constitutive relation chosen for the pressure within the tissue by Arciero et al. [11] was

$$p(\rho) = k\ln(\rho / \rho_{\text{unstressed}}), \tag{2}$$

where $\rho_{\text{unstressed}}$ is a parameter described in Table 1. Thus with Eq. 2 and setting $p = -F$ at the boundary [11,47], we obtain the boundary condition

$$\rho = \rho_{\text{unstressed}} e^{-F/k}, \qquad \text{on } \partial\Omega_1^t. \tag{3}$$

The constitutive relation in Eq. 2 is also the cause for the appearance of the Laplacian in Eq. **1**, hence the Laplacian does not arise from any underlying diffusion process or Brownian motion and k/b should not be interpreted as a diffusion constant (see Supporting Information S2 File or Arciero et al. [11]).

An additional Stefan moving boundary condition is given by

$$\mathbf{v}\cdot\mathbf{n}_1 = \left(-\frac{k}{b}\frac{1}{\rho_{\text{unstressed}}}e^{F/k}\nabla\rho\right)\cdot\mathbf{n}_1, \qquad \text{on } \partial\Omega_1^t, \tag{4}$$

where $\mathbf{v}(\mathbf{x},t)$ is the velocity of the layer and $\mathbf{n}_1(\mathbf{x},t)$ is the outward unit normal to the tissue boundary (see Supporting Information S2 File or Arciero et al. [11]).

On the edge of the computational domain $\partial\Omega_2$, we assume that there is no flux of cells, i.e. cells are unable to move beyond this boundary, and so we have the Neumann boundary condition

$$\nabla\rho\cdot\mathbf{n}_2 = 0, \qquad \text{on } \partial\Omega_2, \tag{5}$$

where $\mathbf{n}_2(\mathbf{x},t)$ is the outward unit normal to the edge of the computational domain. We note that in our simulations, the computational domain $\partial\Omega_2$ is large enough so that the tissue boundary $\partial\Omega_1^t$ is never close to $\partial\Omega_2$.

By segmenting cells in confocal images of the epithelial layer of a representative animal cap explant labeled with a GFP-membrane tag, we measured the tissue density in the epithelial layer at the initial imaging time point to be 0.0047 cells/μm^2, so we take the initial condition to be

$$\rho(x,0) = 0.0047, \qquad \text{in } \Omega^0. \tag{6}$$

In the original model of Arciero et al. [11], the growth term q was included in Eq. 1 to model the volumetric proliferative growth which is typical for spreading sheets of cultured cells, such as IEC-6 or MDCK cells, and taken to be logistic growth. However, the cleavages in early *Xenopus* embryonic tissues are reductive, so migration of explants does not depend on volumetric growth provided by cell proliferation [45]. Therefore, we modify q in Eq. 1 to represent material added through other means, such as active cell shape change or radial intercalation in the epithelial layer.

We observe that since there is a finite number of cells in the initial explant and since tissue mass is conserved, there cannot be unconstrained material growth in the visible epithelial layer.

Furthermore, we estimate that there is at most a 50% increase in number of cells on the surface of the initial explant from either active shape changes or intercalated cells from the lower mesenchymal layer, which is a reasonable upper bound based on the estimate that no more than 20% of the surface cells in the embryo come from the deep mesenchymal layer [38]. Thus, after testing various possible growth functions for qualitative fits to explants' change in area over time, we use a mass-limited density-dependent logistic growth function to describe this addition of visible material into the surface layer,

$$q = q(\rho, m) = \begin{cases} \alpha\rho\left(1 - \rho / \tilde{\rho}\right), & m < \dfrac{m_0}{2}, \\ 0, & m \geq \dfrac{m_0}{2}, \end{cases} \tag{7}$$

where α is a parameter described in Table 1, $\tilde{\rho}$ is the limiting density assumed to correspond to a compressed tissue such that $\tilde{\rho} = \rho_{\text{unstressed}} e^{F/k}$ [11], *m(t)* is the cumulative amount of mass that has been added to the visible surface layer through time *t*, and m_0 is the initial amount of mass.

Details of the numerical method implemented to solve the model equations may be found in Arciero et al. [11], and the output of a toy simulation is shown in Fig 2C.

Parameter Estimation Using Approximate Bayesian Computation (ABC)

Equipped with a mathematical model of collective cell migration in *Xenopus* ectoderm during gastrulation to tailbud stages and kinematic data extracted from time-lapse images, we aim to infer material properties of the tissue that are not directly measurable as well as predict tissue behavior in new situations via estimating model parameters. We use a Bayesian framework, the approximate Bayesian computation (ABC) rejection method [33,34], to determine probability distributions for the model parameters for an individual tissue explant; see Fig 2E for a flow chart depicting this process. The results for the individual explants from our model building set of explants are then compared to

determine whether there is a difference in parameter values for explants of various sizes (Fig 1C). A set of explants is reserved for our test set to examine the predictive power of the mathematical model.

Estimating parameters for our model in a deterministic manner appeared infeasible as preliminary parameter space exploration hinted that our model had parameter structural non-identifiability, or functionally related model parameters [48]. While parameter identifiability has been explored extensively in the case of ordinary differential equations, which involves studying issues such as whether different parameter sets give rise to different model predictions, it has not been studied as much in partial differential equation models [49]. Thus, due to our preliminary parameter space exploration analysis and aware that other partial differential equation models that are similar to our collective cell migration model have shown non-identifiability relations between parameter values [50], we use ABC rejection because it allows for quantification of the uncertainty of our parameter estimates. Furthermore, since we aim to compare multiple data sets, being able to quantify uncertainty in a single data set permits us to analyze the then more complex problem of comparing estimated parameter values among multiple data sets.

To use ABC rejection, the first step is to specify a prior distribution for each of the parameters. This prior distribution is our best educated guess as to what the landscape of the parameter value looks like. The parameter values to be estimated are F, k, b, α, and $\rho_{\text{unstressed}}$ (Table 1), but since three of these parameters, F, k, and b, appear in the model equations only as the ratios F/k and k/b, we cannot uniquely determine each parameter and thus only consider them in the grouped ratios. How we chose the prior distribution for each parameter was based on: previous parameter estimates for IEC-6 cells and MDCK cells from Mi et al. [14] and Arciero et al. [11], knowledge of parameter values that can cause simulations of the mathematical model to leave the computational domain, and knowledge of *Xenopus* ectoderm proliferative behavior and average cell size, and thus tissue density. Hence, the prior distribution for each parameter is chosen to be a broad uniform distribution $U(a,b)$ with probability

density function defined by $f(x) = 1/(b\text{-}a)$ for $x \in [a, b]$, and 0 otherwise, where $F/k \sim U(0,1.5)$ [dimensionless], $k/b \sim U(500,5000)$ [$\mu m^2/h$], $\alpha \sim U(0,1)$ [h^{-1}], and $\rho_{\text{unstressed}} \sim U(1000,2000)$ [cells/μm^2].

The second step of ABC rejection is to sample randomly from the distribution of each of the parameters. We obtain a set of parameters $\{F/k, k/b, \alpha, \rho_{\text{unstressed}}\}$, which we use in a simulation of the mathematical model. Quantification of how well the numerical simulation corroborates with experimental data is determined by calculating the sum of the mean-squared difference between the observed and simulated tissue edge positions and the mean-squared difference between the observed and simulated density ratios.

For the tissue edge positions, the minimum distance from each computational edge point (indexed by n) to every line segment that connects the points along the experimental edge is found for the jth image in the time-lapse sequence, denoted d_n. (In general, there were more points along the experimental edge than the computational edge.) The square root of the average of the squares of these minimum distances is calculated and denoted D_j, where

$$D_j = \left(\sum_{n=1}^{N} \frac{d_n^2}{N} \right)^{1/2}, \tag{8}$$

where index n denotes the computational points counted along the edge and N is the total number of these points. Summing over all time points gives the distances error term

$$z_d = \sum_{j=1}^{t_{\text{end}}} D_j. \tag{9}$$

For the density ratios, at each pixel we approximate the experimental density ratio, denoted as $\xi_{\text{exp},j}$, from Eq. 16 (Materials and Methods Section) with the deformation gradient Eq. 18 for the jth (corresponding with ρ_r) and $(j+\delta)^{\text{th}}$ (corresponding with ρ_s) images in the time-lapse sequence, where δ denotes the frame increment between the pair of images. To limit the impact of inelastic mechanical events such as cell rearrangement or cell division, e.g. cases where tissue position becomes

discontinuous, we limit the time between the pairs of registered images. δ is chosen to be the smallest frame increment in which changes between images can be detected, and in our case, δ=5 (Supporting Information S1 File). Since the size of the matrix $\xi_{\text{exp},j}$ may not be the same size as the computational grid (chosen for computational efficiency), we first linearly interpolate the experimental density ratio values at the computational grid nodes using the MATLAB (The MathWorks, Natick, MA) function `interp2`. The computational density ratio is denoted $\xi_{\text{comp},j}$, which is computed at each grid node by taking the ratio of the density from the jth time point (corresponding with ρ_r) and the $(j+\delta)^{\text{th}}$ time point (corresponding with ρ_s). In other words, to compute $\xi_{\text{comp},j}$ where $\rho(x,y,t)$ is the computational density for each element (ℓ_1, ℓ_2) in the grid,

$$\xi_{\text{comp},j} = \frac{\rho(\ell_1, \ell_2, j)}{\rho(\ell_1, \ell_2, j+\delta)}. \tag{10}$$

The square root of the average of the squares of the differences between the experimental and computational density ratios is

$$P_j = \left(\sum_{\ell_1=1}^{L_1} \sum_{\ell_2=1}^{L_2} \frac{\left(\xi_{\text{comp},j} - \xi_{\text{exp},j}\right)^2}{L_1 L_2} \right)^{1/2}, \tag{11}$$

where $\xi_{\text{comp},j}$ and $\xi_{\text{exp},j}$ are evaluated at grid node (ℓ_1, ℓ_2), and the size of the computational grid is $L_1 \times L_2$. Summing over all time points (minus frame increment δ) gives the density ratios error term

$$z_p = \sum_{j=1}^{t_{\text{end}}-\delta} P_j. \tag{12}$$

We take the total error to be

$$z = z_d + w z_p, \tag{13}$$

where we use weight w = 1000 to ensure that the distances error term z_d and density ratios error term z_p are approximately the same order. If the simulated explant migrates outside the computational domain,

the error is taken to be "NaN" in our numerical code to signify an extremely poor fit since the entire explant remains within the domain during the time-lapse sequence. Furthermore, if the density in the center of the explant increases in the numerical simulation, the error is also taken to be "NaN" since explants thin out in our experiments.

We collected 10,000 parameter sets that result in total error (Eq. 13) less than or equal to a tolerance threshold of 1500 for each explant. The large computational expense of running this parameter set collection on a supercomputer was over 100,000 CPU hours and made the gathering of thousands or millions of more samples not feasible. We retained all of the 10,000 parameter sets to be posterior samples to represent the distribution of parameter values that are optimal rather than a percentage of the 10,000 due to the sample size. These posterior distributions are the updated best estimates for what the landscape of each parameter value looks like. Some of the analysis below was done with the smallest 20% (in terms of the total error) of the 10,000 parameter sets.

For each of the eighteen explants, we used the ABC rejection algorithm to obtain posterior parameter value distributions for F/k, k/b, α, and $\rho_{\text{unstressed}}$. To visualize the posterior parameter value distributions for the four parameters, we projected the distributions down to one and two dimensions, as in Fig 3A which displays the results for one explant. Along the diagonal are smoothed histograms for the one-dimensional projections of the individual parameters. Below the diagonal are the two-dimensional projections of pairs of parameters. Darker shaded areas correspond with higher frequency where higher frequency corresponds with the parameter value being sampled more often.

Fig 3. Results of approximate Bayesian computation rejection method and statistics results. (A) Triangle plot of posterior distributions obtained by the ABC rejection method for Explant #8. Along the diagonal, the plot shows smoothed histograms for the 1-D projections of the parameters F/k, k/b, α, and $\rho_{\text{unstressed}}$. The lower part of the triangle shows the 2-D histograms for all pairs of these four parameters. The darker shades in the 2-D histograms denote more frequently occurring values of parameters, and the dashed vertical and horizontal lines denote the most frequently occurring values of each parameter. The purple circle indicates the mean parameter sets and the blue, red, and yellow asterisks indicate the parameter sets with the smallest errors, respectively. 10,000 parameter sets were used. Cf. Supporting Information S1 Figure for the triangle plot colored in terms of the error (Eq. 13)

instead of frequency.
(B) Correlation coefficient for each pair of parameters for all 18 explants. The average correlation coefficient for each pair of parameters over all the explants is displayed on the right side of the plot. The smallest 20% (in terms of the total error) of the 10,000 parameter sets were used per explant.
(C) Boxplots of the 1-D projections of the posterior distributions of the parameters F/k, k/b, α, and $\rho_{\text{unstressed}}$ for all 18 explants, grouped by region Ia, Ib, II, and III (see Supporting Information S2 Figure for non-grouped boxplots). The compact letter display (CLD) and colors reflect the results for Tukey's multiple comparisons test; in particular, boxplots labeled with the same letter were considered not statistically different (based on significance level 0.05). Boxplots labeled with *** were statistically significantly different than all the other boxplots. Coloring was chosen to reflect groups of boxplots that were labeled similarly. The plus signs indicate outliers, the lines within the boxes indicate the median, and the length of the boxplot indicates the interquartile range. 10,000 parameter sets were used per explant. Cf. Supporting Information S2 Figure for the non-grouped boxplots.

Model parameters describing explant spreading reveals an inverse relationship between F/k and k/b, which implies non-identifiability, and less distinct relationships between the other pairs of parameters, which may imply identifiability or weak non-identifiability (Fig 3A). This relationship is further indicated in Supporting Information S1 Figure, which colors the results of the triangle plot in Fig 3A by the value of the error (Eq. 13) instead of frequency. To verify the association between the different parameters, we calculated the correlation coefficient for each pair of parameters for each explant (Fig 3B). We found that F/k and k/b were strongly negatively correlated, pair k/b and α and pair α and $\rho_{\text{unstressed}}$ were weakly correlated, and the other parameter pairs were moderately correlated.

We also calculated 95% confidence intervals for the one-dimensional projections of the posterior distributions for each parameter for each explant; see Supporting Information S1 Table. We observe that there appears to be trends in the mean parameter value versus the initial area of the explant, but to verify this we ran ANOVA tests. Using 10,000 parameter sets per explant and grouping them into regions Ia, Ib, II, and III (in other words, the parameter sets for Explants #1-5 were combined into one set, the parameter sets for Explants #6-12 were combined into one set, etc.), we determined that, for all the parameters F/k, k/b, α, and $\rho_{\text{unstressed}}$, the p-value $< 10^{-29}$. This implies that there is statistically significant evidence that not all of the average parameter values were the same for the four

groups of explants. We repeated this analysis without grouping the explants into regions but instead individually comparing each, and found that the *p*-values were even smaller.

Additionally, we ran Tukey's multiple comparisons test to compare the average parameter values of the eighteen explants, grouped into the four regions and individually, and to determine which showed statistically significant differences. Figure 3C shows boxplots of the one-dimensional projections of the posterior distribution for each parameter for the explants grouped into the four regions. The boxplots are labeled with a compact letter display (CLD) such that boxplots labeled with the same letter were considered not statistically different (based on significance level 0.05). In Supporting Information S2 Figure, boxplots of the non-grouped explants are shown. We found that α and $\rho_{\text{unstressed}}$ are not affected much by the initial area of the explant, indicated by fewer separate groupings (denoted by more overlapping letters in the CLD as well as boxplots of the same color), but *F/k* and *k/b* are affected.

To further test whether estimated values of *F/k*, *k/b*, α, and $\rho_{\text{unstressed}}$ are associated with the initial area of an explant, we ran a linear regression *t*-test with the mean values of each parameter from the one-dimensional projection of the posterior distribution for each explant. With *p*-value = 0.02 for *F/k*, *p*-value = 0.002 for *k/b*, and *p*-value = 0.0004 for $\rho_{\text{unstressed}}$, there is evidence that there is a relationship between the parameter value and initial area of the explant depending on the significance level chosen. With *p*-value = 0.17 for α, there is little evidence that there is a relationship.

The slopes of the sample regression line for the mean value of *F/k* vs. initial area and the sample regression line for the mean value of *k/b* vs. initial area were both positive while the slope of the sample regression line for the mean value of $\rho_{\text{unstressed}}$ vs. initial area was negative. This suggests a general trend that both *F/k* and *k/b* increase as the initial area increases and $\rho_{\text{unstressed}}$ decreases as the initial area increases. The slope of the sample regression line for the mean value of α vs. initial area was close to 0. Furthermore, if we assume that the stretching modulus *k* is constant for each explant,

which is a realistic expectation since explants are isolated from similar locations, then these results imply that for larger explants the forces produced by the lamellipodia (F) are stronger and the adhesion forces (b) are weaker. A similar relationship between the lamellipodia force and the size of the initial explant was also observed in primary mouse keratinocyte cell colonies [51,52], where traction stresses increase with colony radius. In addition, from the inverse relationship between F/k and k/b in the triangle plot (Fig 3A), we predict that if adhesion is increased, then the measured traction force will also increase.

Prediction

To determine whether the posterior distributions for the parameter values of F/k, k/b, α, and $\rho_{\text{unstressed}}$ were robust, we took the parameter set that resulted in the smallest error (Eq. 13) for an explant in our model building set and used it in a simulation of another explant of similar size from the same region from our test set of explants (Fig 4A-D) or differently sized from a different region (Fig 4E-F). We found that for similarly sized explants that have similar average changes in area over time $\Delta A/\Delta t$, the error (Eq. 13) remained small for the explants in the test set. For differently sized explants, the error was large for the explants in the test set. Furthermore, we observed that similarly sized explants that do not have similar average changes in area over time $\Delta A/\Delta t$, such as the areas of Explant #8 and Explant B which are represented in the third and fourth lowest curves in Fig 1D, also resulted in larger errors for explants in the test set. Hence, this indicates that our posterior distribution results are robust since explants of similar size will have similar model parameters that best fit the experimental data and explants of different size will not have similar model parameters that best fit the experimental data. For comparison, simulations for Explants #1, 8, 14, and 17 are shown in Supporting Information S3 Figure and the Supporting Information Videos.

Fig 4. Simulation of tissue migration for various test explants using estimated parameter values from similarly sized or differently sized explants.
Progression of *Xenopus* animal cap explant tissue migration at 95 minute time intervals for test

explants A, B, C, and D with the parameter set that resulted in the smallest error (Eq. 13) from the posterior distributions for a similarly sized explant from the same region (A-D) or a differently sized explant from a different region (E-G). In the top panel, the computed edge from the mathematical model is represented by a solid dark red curve and the experimental edge is represented by a dotted light yellow curve. The bottom panel shows the absolute value of the density ratio at each grid node (where both density ratios are nonzero) of the experimental data between the given time point and 25 minutes later minus the computed density ratio. Scale bar: 500 μm. See the Supporting Information Videos for time-lapse sequences of these still images.
(A) Test Explant A [Region Ia] (initial area 0.16 mm^2) using parameters for Explant #1 [Region Ia] (initial area 0.14 mm^2): $F/k = 0.6101$, $k/b = 510\ \mu m^2/h$, $\alpha = 0.9479\ h^{-1}$, $\rho_{unstressed} = 1404$ cells/μm^2. The total error with these parameters for Test Explant A is 1091 while for Explant #1 it is 1122.
(B) Test Explant B [Region Ib] (initial area 0.44 mm^2) using parameters for Explant #8 [Region Ib] (initial area 0.45 mm^2): $F/k = 0.9059$, $k/b = 510\ \mu m^2/h$, $\alpha = 0.9492\ h^{-1}$, $\rho_{unstressed} = 1313$ cells/μm^2. The total error with these parameters for Test Explant B is 1109 while for Explant #8 it is 820.
(C) Test Explant C [Region II] (initial area 1.26 mm^2) using parameters for Explant #14 [Region II] (initial area 1.12 mm^2): $F/k = 0.7948$, $k/b = 635\ \mu m^2/h$, $\alpha = 0.9410\ h^{-1}$, $\rho_{unstressed} = 1530$ cells/μm^2. The total error with these parameters for Test Explant C is 869 while for Explant #14 it is 774.
(D) Test Explant D [Region III] (initial area 2.23 mm^2) using parameters for Explant #17 [Region III] (initial area 2.14 mm^2): $F/k = 0.8295$, $k/b = 950\ \mu m^2/h$, $\alpha = 0.8963 h^{-1}$, $\rho_{unstressed} = 1752$ cells/μm^2. The total error with these parameters for Test Explant D is 933 while for Explant #17 it is 834.
(E) Test Explant D [Region III] (initial area 2.23 mm^2) using parameters for Explant #1 [Region Ia] (initial area 0.14 mm^2): The total error for Test Explant D is 1486.
(F) Test Explant D [Region III] (initial area 2.23 mm^2) using parameters for Explant #8 [Region Ib] (initial area 0.45 mm^2): The total error for Test Explant D is 1136.
(G) Test Explant D [Region III] (initial area 2.23 mm^2) using parameters for Explant #14 [Region II] (initial area 1.12 mm^2): The total error for Test Explant D is 1140.
(H) Average change in area over time ($\Delta A/\Delta t$), calculated as the slope of the regression line of area measurements over time, for *Xenopus* animal cap explants of different initial sizes. The explants are grouped by initial area into subgroups Ia, Ib, II, and III, indicated by the dashed lines. The blue filled-in circles correspond with the explants included in our model building set and the red circles correspond with the explants included in our test set. The length of time was 10 hours.

Discussion

Time-lapse imaging of tissue explant migration generates complicated datasets that are information-rich. Here, we present a formal methodology for extracting mechanical mechanisms of tissue spreading by integrating complex image information with a mathematical model of cell migration. This approach identifies key differences between experimental groups of differently sized embryonic tissue explants. In order to directly relate the experimental kinematics of spreading of multiple tissue explants to mechanical properties of the tissue, we focused on one mathematical model,

the previously-developed Eulerian mechanical model of Arciero et al. [11], that represents forces involved in migration within a 2D elastic continuum. In this modeling framework, extended here to represent *Xenopus* animal cap epiboly, we can directly couple experimentally derived kinematic data to model parameters that correspond to physical properties in the tissue.

Since cell nuclei positions were not recorded in our time-lapse images, we mapped strain across the tissue and used changes in strain to estimate local changes in tissue density. While in the current study we used variations in pigment to calculate strain and time-dependent changes in density, any landmark-rich image, for example, random textures of sub-cellular organelles, dye, or fluorescence, could be used to obtain density ratios. Furthermore, our assumption that the tissues are elastic for the image registration and the mathematical model, could be relaxed to accommodate more realistic constitutive models such as viscoelasticity or viscoplasticity [53–55]. Since strain mapping techniques do not depend on free edges, the strain mapping techniques presented here can also be extended beyond tissues in two dimensions to analyze more complex structures, organs, and whole organisms.

Previous studies of tissue spreading that used methods developed by Arciero et al. [11] and Mi et al. [14] analyzed two different cultured cell lines, rat intestinal epithelial cells (IEC-6) and Madin-Darby canine kidney (MDCK) epithelial cells, and estimated model parameters for individual experiments. While these studies report one parameter set that matches a single set of experimental data, we show here that while there may be a single "optimal" parameter set for each set of experimental data, due to the inverse relation between model parameter ratios F/k and k/b, there is a wide range of "close to optimal" parameter sets. Thus, to compare between different sets of experimental data, we needed to implement a robust statistical analysis capable of accounting for the uncertainty in the model parameter estimates.

The approximate Bayesian computation (ABC) rejection method was utilized to collect multiple parameter sets that, when used in a computer simulation of the mathematical model, resulted

in sufficiently good fits to the experimental data. Fit was determined via an error function that calculated the difference between the model generated boundary and strain field and image-based measurements of tissue boundary and density changes throughout the simulation, rather than just matching a single end time point. Hence, we used as much as possible of the available image dataset to estimate physically-relevant parameter values. Statistical analysis then allowed us to examine the difference in means in multiple parameter set distributions for the multiple experiments of explants of varying sizes. Using this methodology, we found that there are interdependencies between parameters F/k and k/b. We also detected trends that for larger explants, adhesion forces (b) are weaker and forces produced by lamellipodia (F) are stronger if we assume that the stretching modulus k is fixed among explants since they are comprised of the same tissue. We used the same prior uniform distribution for each of the explants (Fig 2D) and observed that in general, more simulations needed to be run to collect 10,000 parameter sets that resulted in an error (Eq. 13) under the required threshold for smaller explants compared to larger explants (Supporting Information S5 Figure). The parameter values that fit the experimental data the best for smaller explants appear to be more on the extremes of the prior uniform distribution than for larger explants.

Large computational expenses limited the number of parameter sets gathered for each explant to 10,000 for the ABC method as well as the size of our model building set to 18 of 41 available explants in our experimental data set. However, we observed that the parameter values found were robust among similarly sized explants (Fig 4). Indeed, for explants with similar average change in area over time ($\Delta A/\Delta t$), simulations with a parameter set that resulted in a small error (Eq. 13) for an explant in the model building set also resulted in a small error for a similarly sized explant in the test set. Thus we would expect that the trends we discovered would be the same if we analyzed more explants in the future, taking into account embryo to embryo variation in stiffness [56], clutch to clutch variation [57], and possibly environmental variations such as room temperature that might affect rates and

deformation maps of tissue spreading [58]. We also propose adding the velocity or the change in area over time as another input to the error function Eq. 13 as it appears to have an effect on estimating parameters (Fig 1C and 4). Gathering more parameter sets through the ABC method will also allow for the probability distributions of the parameters to become more precise. Further investigation of the estimated set of model parameters would require the ability to measure one of the parameters directly from experiments, reducing the total number of parameters.

The probability distributions provide ranges for parameters F/k and k/b that can be compared to direct measurements or may be manipulated experimentally (Fig 4). Since parameters F, k, and b appear only as the ratios F/k and k/b in the mathematical model, to estimate values for each of those parameters individually we would need a measurement of at least one of the quantities. From previous studies we estimate that the stiffness of the ectodermal epithelial layer of *Xenopus* gastrula is approximately 1.2 mN/m [53,57,59]. Using this value as an approximation for k, keeping in mind that our explants included both the epithelial and mesenchymal layers, we predict that traction forces at the explant edge (F) range from 0.59–0.91 nN/μm and that adhesion (b) ranges from 0.0004–0.0007 nNh/μm^3 (using the minimum lower bound and maximum upper bound of the 95% confidence intervals in Supplementary Table 1).

Though our focus in this study was on analyzing multiple experiments with one mathematical model, our work suggests future extensions to the Eulerian model include incorporating heterogeneity within the tissue and in its environment in order to model geometrically and mechanically complex tissues in diverse microenvironments. For example, complex features that might improve agreement with asymmetrically spreading tissue include cell layer viscosity, anisotropic tissue stiffness, patterned extracellular matrix, and distinct material models for substrate non-linear elasticity. Furthermore, since the *Xenopus* animal cap explants consist of a single epithelial layer and multiple layers of deep

mesenchymal cells, models that explicitly represent interactions between cells in these two tissues might better account for their integrated spreading movements.

Recent years have witnessed a deluge of high quality image data from confocal and light-sheet microscopy from both *in vivo* and *ex vivo* models of tissue morphogenesis, but efforts to integrate such kinematic descriptions have lagged behind systematic methods that are capable of extracting both patterns of force production and patterns of tissue mechanical properties. Our Eulerian mechanical model with approximate Bayesian computation achieves that goal, suggesting future mechanical modeling approaches based on density or density ratios could be directly integrated with kinematic data extracted using automated methods of strain mapping for comparing multiple experiments. These efforts will lead to more rapid analysis of morphogenetic movements and the improved correlations between molecular scale perturbations and phenotypic change.

Materials and Methods

Embryos and sample preparation

Eggs were collected from *Xenopus laevis* frogs and fertilized *in vitro* using standard methods [45]. After fertilization eggs were dejellied and cultured in 1/3 X Modified Barth's Saline (MBS) [60]. Embryos used to analyze intercalation were transferred to a 3% ficoll solution in 1X MBS and were injected (Harvard Apparatus) with approximately 1ng mRNA encoding a membrane tagged GFP (mem-GFP [61]) at the two cell stage, after which they were returned to 1/3 X MBS. Embryos were raised to approximately Stage 10 [37] and were transferred to explant culture medium (Danalchik's for Amy; DFA) [62]. Embryos were devitelinized with forceps and the animal cap ectoderm was microsurgically removed using hair tools (Fig 1A-B). Tissue explants were cultured on either a petri dish (Fisher) or in a glass-bottomed chamber coated with human plasma fibronectin (Roche Molecular

Biochemicals) in DFA supplemented with antibiotic/antimycotic (Sigma). Fibronectin coated substrates were created by filling the chamber with a concentration of 25 μg/mL human Fibronectin (Roche) at 4°C overnight. Explants were allowed to adhere for 30 minutes prior to imaging. Eggs used in this project came from a colony of African Claw-Toed Frogs, *Xenopus laevis*, maintained at the University of Pittsburgh according to provisions established by the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh (Protocol #12020250).

The embryonic tissue explants generally spread symmetrically outward over their fibronectin-coated substrate in our experiments. Any variation in spreading was likely due to unseen substrate defects or anisotropy in fibronectin substrate density but did not affect the overall spreading rate of explants. As expected, reducing the fibronectin concentration below 1.0 μg/mL reduced the spreading rate (Supporting Information S4 Figure).

Imaging

Stereoscope images of tissue explants spreading were collected using a CCD camera (Scion Corporation) mounted on a stereoscope (Stemi 2000; Zeiss) (Fig 1D; first row). Multiple explants could be imaged in the same dish using a motorized XY stage (Marzhauser and Ludl) controlled through microscope automation and image acquisition software (μ-Manager [63]). In order to determine cell density, high resolution confocal images of membrane tagged GFP in live samples were collected using an inverted compound microscope equipped with a laser-scanning confocal scanhead (SP5; Leica Microsystems). In some cases, a stack of images over a z-range of 50 to 100 μm were collected and combined into a single image using maximum intensity projection prior to analysis.

Image Analysis

Subsequent image analysis of time-lapse sequences were performed using ImageJ ([64]; available for download at http://imagej.nih.gov/ij/) and Fiji ([65]; available for download at http://fiji.sc/) as described below. Cell boundaries were identified semi-automatically using SeedWater

Segmenter ([66]; available for download at https://my.vanderbilt.edu/shanehutson/software/seedwater-segmenter/) in order to estimate cell density.

Tissue edge boundaries were identified from single stereoscope images using the Level Sets plugin in Fiji [65], which is an image segmentation technique based on partial differential equations (Fig 1D; second row). The error is expected to be less than 5 pixels, which corresponded to no more than 2 cell widths (approximately 40 μm). To calculate the average areal spreading rate, the area within this boundary was determined at each time point. These areas were then fit using linear regression. The slope of the resulting regression line was defined as the average change in area over time ($\Delta A/\Delta t$) (Fig 1C).

Strain Mapping Method

Time-lapse sequences provide a rich set of information on the movement of tissues. In addition to edge detection, digital image correlation can be used to track individual features such as cell nuclei, cell membranes, or even textural features such as pigment granule distribution that do not change significantly between sequential images [42,67]. By tracking these features, a "translation" map or Eulerian description of flow can be produced showing where parcels of tissue move over time [43]. This map can then give an estimate of local density change.

Elastic registration between two images in a time-lapse sequence separated by a fixed amount of time represents the kinematic transformation between the two images and makes minimal assumptions about the mechanical properties of the materials involved. The usefulness of the registration, i.e. that it reflects linear deformations in the ectoderm, relies on additional assumptions based on the biology of *Xenopus* embryos. Since cell division, rearrangement, and radial intercalation events are typically slow, these processes do not disrupt the pigment patterns observed at the apical face of the ectoderm.

Since we are examining a short-term analysis of strain in time, we assume that the *Xenopus* tissue is in a quasi-static equilibrium and mass is added very slowly between each pair of images, and thus we may assume that there is local conservation of mass. Letting dV_r be the infinitesimal volume in the reference material coordinates and dV_s be the corresponding infinitesimal volume in the spatial coordinates, the change of volume due to deformation [68] can be written as

$$dV_s = \det \mathbf{F}\ dV_r, \tag{14}$$

where $\mathbf{F}$ is the deformation gradient defined as $\mathbf{F}_{mn}(\mathbf{X},t) = \dfrac{\partial \mathbf{x}_m(\mathbf{X},t)}{\partial \mathbf{X}_n}$, where $\mathbf{X}=(\mathbf{X}_1,\mathbf{X}_2)$ is the (x,y)-coordinate positions in the first still image in pixels and $\mathbf{x}=(\mathbf{x}_1,\mathbf{x}_2)$ is the (x,y)-coordinate positions in the second still image.

Eq. 14 implies that the Jacobian det $\mathbf{F}$ measures the ratio of the volumes. The conservation of mass equation

$$\rho_s\ dV_s = \rho_r\ dV_r, \tag{15}$$

thus implies that, locally,

$$\det \mathbf{F} = \frac{\rho_r}{\rho_s}. \tag{16}$$

Hence, if we can calculate the deformation gradient $\mathbf{F}$, we can obtain an estimate of the local change in density.

To perform digital image correlation on image pairs of pigmented ectoderm tissue and calculate strain, or local deformation, we use a texture mapping strategy with the ImageJ software plugin bUnwarpJ, which is used for elastic and consistent image registration ([69]; available for download at http://fiji.sc/BUnwarpJ). By using differential image correlation techniques [70], we calculate deformation of the tissue via the translation map (Fig 1D; third-sixth rows). For details on the strain

mapping method discretization to obtain numerical estimates of the x-strain ε_{xx}, y-strain ε_{yy}, xy-strain ε_{xy}, yx-strain ε_{yx} (which is equal to ε_{xy}), and displacement gradient $\nabla\mathbf{u}$, please see the Supporting Information S1 File.

Since the gradient of the displacement vector is defined as

$$\nabla\mathbf{u}(\mathbf{X},t) = \mathbf{F}(\mathbf{X},t) - \mathbf{I}, \tag{17}$$

where $\mathbf{I}$ is the identity matrix, using the discretization of the displacement gradient $\nabla\mathbf{u}(i,j)$ we numerically approximate the deformation gradient at each pixel (i,j) as

$$\mathbf{F}(i,j) = \nabla\mathbf{u}(i,j) + \mathbf{I}. \tag{18}$$

Taking the determinant for each i and j, Eq. 18 gives a numerical approximation of the density ratio in Eq. 16 at each pixel (Fig 1D; last row).

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Supporting Information

Supporting Files

S1 File. Strain mapping method discretization.

Here we describe the implementation of the strain mapping method to calculate the deformation of a tissue via estimates of the x-strain ε_{xx}, y-strain ε_{yy}, xy-strain ε_{xy}, yx-strain ε_{yx}, and displacement gradient $\nabla\mathbf{u}$ between two images in a time-lapse sequence.

For images of width n pixels and height m pixels, the properties of each pixel can be represented by an entry in an $m\times n$ matrix. For each pair of images from the time-lapse sequence, let $\mathbf{X}=(\mathbf{X}_1,\mathbf{X}_2)$ be the (x,y)-coordinate positions in the first still image in pixels and $\mathbf{x}=(\mathbf{x}_1,\mathbf{x}_2)$ be the (x,y)-coordinate positions in the second still image. The top left corner of an image is the origin, and x increases from left to right and y increases from top to bottom. The entries of $\mathbf{X}$ are therefore defined by $\mathbf{X}_1(i,j)=j-1$ and $\mathbf{X}_2(i,j)=i-1$ for $i=1,2,\ldots,n$, and $j=1,2,\ldots,m$.

We mask the pair of images so that registration occurs only in the actual location of cells. We then initialize bUnwarpJ to calculate the coefficients of the cubic B-spline map β that defines the transformation $(\mathbf{X}_1, \mathbf{X}_2) \xrightarrow{\beta} (\mathbf{x}_1, \mathbf{x}_2)$ [69]. Initializing bUnwarpJ again, we apply β to $\mathbf{X}$ by converting the transformation to "raw" data, which reports the elastically-mapped position of each pixel in the first image to the "fitted" position in the second image. We obtain $\mathbf{x}$, the mapped position of each pixel from the first image to its position in the second image, in pixels. Note that pixels outside of the mask will be mapped as well, but we will remove this extraneous data after we have obtained the strains. Next, we calculate the displacement vector $\mathbf{u}$ by

$$\begin{aligned}
\mathbf{u}_1(i,j) &= \mathbf{x}_1(i,j) - \mathbf{X}_1(i,j), \qquad i=1,2,\ldots,n, \quad j=1,2,\ldots,m,\\
\mathbf{u}_2(i,j) &= \mathbf{x}_2(i,j) - \mathbf{X}_2(i,j), \qquad i=1,2,\ldots,n, \quad j=1,2,\ldots,m.
\end{aligned} \tag{19}$$

The engineering, or Cauchy, strain is defined as

$$\varepsilon = \frac{\Delta L}{L_0} = \frac{L-L_0}{L_0}, \tag{20}$$

where ΔL is the change in length of the tissue, L_0 is the original length, and L is the current length. The displacement vector $\mathbf{u}$ is converted into x-strain, y-strain, xy-strain, and yx-strain by

$$\begin{aligned}
\varepsilon_{xx}(i,j) &= \frac{\mathbf{u}_1(i,j)-\mathbf{u}_1(i-1,j)}{\mathbf{X}_1(i,j)-\mathbf{X}_1(i-1,j)}, \qquad i=2,3,\ldots,n, \quad j=1,2,\ldots,m,\\
\varepsilon_{xy}(i,j) &= \frac{\mathbf{u}_1(i,j)-\mathbf{u}_1(i,j-1)}{\mathbf{X}_2(i,j)-\mathbf{X}_2(i,j-1)}, \qquad i=1,2,\ldots,n, \quad j=2,3,\ldots,m,\\
\varepsilon_{yx}(i,j) &= \frac{\mathbf{u}_2(i,j)-\mathbf{u}_2(i-1,j)}{\mathbf{X}_1(i,j)-\mathbf{X}_1(i-1,j)}, \qquad i=2,3,\ldots,n, \quad j=1,2,\ldots,m,\\
\varepsilon_{yy}(i,j) &= \frac{\mathbf{u}_2(i,j)-\mathbf{u}_2(i,j-1)}{\mathbf{X}_2(i,j)-\mathbf{X}_2(i,j-1)}, \qquad i=1,2,\ldots,n, \quad j=2,3,\ldots,m.
\end{aligned} \tag{21}$$

Note that all of the denominators above equal 1 pixel and the shear strains $\varepsilon_{xy} = \varepsilon_{yx}$. At this point, the strains can be visualized to show where there are contractions in the tissue ($\varepsilon < 0$) and where there are dilations ($\varepsilon > 0$) [71].

Using these strain calculations, we can numerically approximate the displacement gradient at each pixel as

$$\nabla \mathbf{u}(i,j) = \begin{pmatrix} \varepsilon_{xx}(i,j) & \varepsilon_{xy}(i,j) \\ \varepsilon_{xy}(i,j) & \varepsilon_{yy}(i,j) \end{pmatrix}, \qquad i = 2,3,\ldots,n \quad j = 2,3,\ldots,m. \tag{22}$$

We found that we could limit numerical boundary effects on the registration by ensuring the tissue was at least 200 pixels from the outer boundary of the image. To ensure the registration between images was detecting movement and not noise, we chose a time interval long enough for movement to be discernible. In our case, a time interval of 25 minutes ensured that the relative change in area between image pairs was on average more than 5%, which would correspond with strain measurements above the noise between images.

S2 File. Mathematical model derivation.

Here we describe the derivation of the mathematical model of single layer cell migration of Arciero et al. [11].

The cell layer is represented as a 2D compressible fluid, and the variable ρ describes the tissue density as a function of position $\mathbf{x}=(x,y)$ and t. The law of conservation of mass,

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) = q, \tag{23}$$

where $\mathbf{v}$ is the velocity of the cell layer, includes the growth term q which may generally depend on space $\mathbf{x}$, time t, or density ρ, and describes the net rate of change in the number of cells within the layer.

Balance of linear momentum implies

$$\rho \frac{\partial \mathbf{v}}{\partial t} + \rho(\mathbf{v} \cdot \nabla)\mathbf{v} = \mathbf{f} + \nabla \cdot \mathbf{T}, \tag{24}$$

where the tensor $\mathbf{T}$ represents the stresses within the cell layer and $\mathbf{f}$ accounts for the force of adhesion of the cell layer to the substrate. $\mathbf{f}$ is the result of the action exerted on a material element by the substrate, i.e. the negative of traction force. It is assumed that the $\mathbf{f}$ is negatively proportional to the cell layer velocity,

$$\mathbf{f} = -b\mathbf{v}, \tag{25}$$

where b is a constant of adhesion. The cell layer is assumed to behave as a compressible inviscid fluid with the constitutive equation

$$\mathbf{T} = -p(\rho)\mathbf{I}, \tag{26}$$

where p is the pressure within the cell layer. The pressure depends on the tissue density and is taken to be positive when cells are compressed and negative when cells are stretched. Assuming acceleration is negligible and substituting Eqs. 25 and 26 into Eq. 24 we obtain the equation

$$b\mathbf{v} = -p'(\rho)\nabla\rho, \tag{27}$$

which is the relation between the velocity of cells and the gradient of tissue density; it resembles Darcy's law describing the flow of fluid through a porous medium.

Substituting Eq. 27 into Eq. 23 results in the governing equation that describes the evolution of tissue density,

$$\frac{\partial\rho}{\partial t} = \frac{1}{b}\nabla\cdot\left(\rho p'(\rho)\nabla\rho\right) + q. \tag{28}$$

In Arciero et al. [11], various constitutive relations for function $p(\rho)$ were considered, but the main relation studied was

$$p(\rho) = k\ln\left(\rho / \rho_{\text{unstressed}}\right), \tag{29}$$

as it gave appropriate behavior at both large and small densities. Substituting the constitutive relation in Eq. 29 into Eq. 28 gives the governing equation

$$\frac{\partial\rho}{\partial t} = \frac{k}{b}\Delta\rho + q. \tag{30}$$

Notice that the Laplacian that appears in the governing equation is due to the constitutive relation chosen for the pressure and hence the governing equation should not be thought of as reaction-diffusion equation.

One boundary condition imposed on the boundary of the cell layer is that there is a constant force per unit length F outward directed against the substrate due to lamellipodia, which requires setting $p = -F$ in Eq. 29 and then solving for ρ, resulting in

$$\rho = \rho_{\text{unstressed}} e^{-F/k}, \qquad \text{on } \partial\Omega_1^t. \tag{31}$$

Another boundary condition imposed on the boundary of the cell layer is a Stefan condition, which describes the speed of the moving edge. This condition comes from Eq. 27 evaluated at Eq. 31 and is

$$\mathbf{v}\cdot\mathbf{n}_1 = \left(-\frac{k}{b}\frac{1}{\rho_{\text{unstressed}}}e^{F/k}\nabla\rho\right)\cdot\mathbf{n}_1, \qquad \text{on } \partial\Omega_1^t. \tag{32}$$

Supporting Tables

S1 Table. 95% confidence intervals for the one-dimensional projections of the posterior distributions for each parameter for each explant.
10,000 parameter sets were used per explant.

Parameter		*F/k* (dimensionless)		*k/b* ($\mu m^2/h$)		α (h^{-1})		$\rho_{unstressed}$ (cells/μm^2)	
Explant	**Initial Area (mm^2)**	**Lower Bound**	**Upper Bound**	**Lower Bound**	**Upper Bound**	**Lower Bound**	**Upper Bound**	**Lower Bound**	**Upper Bound**
1	0.14	0.4949	0.5060	1700	1739	0.4678	0.4793	1528	1539
2	0.23	0.5188	0.5299	1904	1946	0.4514	0.4629	1513	1524
3	0.23	0.4112	0.4223	1937	1983	0.4376	0.4491	1528	1539
4	0.24	0.4156	0.4269	2161	2209	0.4244	0.4358	1523	1535
5	0.26	0.4980	0.5091	1903	1946	0.4585	0.4699	1519	1530
6	0.30	0.4397	0.4517	2262	2311	0.4470	0.4584	1512	1524
7	0.36	0.5084	0.5195	2047	2093	0.4504	0.4618	1515	1526
8	0.45	0.5309	0.5425	2355	2402	0.4419	0.4532	1491	1502
9	0.54	0.6276	0.6391	2653	2700	0.4658	0.4772	1468	1479
10	0.60	0.6630	0.6741	2718	2764	0.4697	0.4811	1460	1471
11	0.64	0.5430	0.5549	2508	2556	0.4497	0.4610	1480	1491
12	0.68	0.6079	0.6197	2624	2672	0.4545	0.4659	1467	1478
13	0.86	0.5499	0.5621	2502	2550	0.4423	0.4538	1482	1494
14	1.12	0.4848	0.4965	2366	2415	0.4357	0.4470	1489	1500
15	1.49	0.4585	0.4704	2318	2366	0.4397	0.4510	1488	1499
16	1.73	0.5779	0.5898	2614	2663	0.4550	0.4663	1469	1481
17	2.14	0.5524	0.5649	2556	2605	0.4419	0.4532	1464	1476
18	2.57	0.7522	0.7621	2943	2987	0.5011	0.5122	1440	1451

Supporting Figures

S1 Figure. Scatterplot of accepted parameter value sets from the approximate Bayesian computation rejection method for Explant #8.
Each circle indicates an accepted parameter value set found using the approximate Bayesian computation rejection method (cf. Figure 3A), and the color of the circle corresponds with its calculated error (Eq. 13).

S2 Figure. Boxplots of the 1-D projections of the posterior distributions of the parameters F/k, k/b, α, and $\rho_{\text{unstressed}}$ for all 18 explants (cf. Fig 3C).
The compact letter display (CLD) and colors reflect the results for Tukey's multiple comparisons test; in particular, boxplots labeled with the same letter were considered not statistically different (based on significance level 0.05). Boxplots labeled with *** were statistically significantly different than all the other boxplots. Coloring was chosen to reflect groups of boxplots that were labeled similarly. The plus signs indicate outliers, the target symbols indicate the median, and the length of the boxplot indicates the interquartile range. 10,000 parameter sets were used per explant. Cf. Fig **3** for the grouped boxplots.

S3 Figure. Simulation of tissue migration for Explants #1, 8, 14, and 17.
Progression of *Xenopus* animal cap explant tissue migration at 95 minute time intervals for Explants #1, 8, 14, and 17 with the parameter set that resulted in the smallest error (Eq. 13). In the top panel, the computed edge from the mathematical model is represented by a solid dark red curve and the experimental edge is represented by a dotted light yellow curve. The bottom panel shows the absolute value of the density ratio at each grid node (where both density ratios are nonzero) of the experimental data between the given time point and 25 minutes later minus the computed density ratio. Scale bar: 500 μm. See the Supporting Information Videos for time-lapse sequences of these still images.
(A) Explant #1 [Region Ia] (initial area 0.14 mm^2): $F/k = 0.6101$, $k/b = 510\ \mu m^2/h$, $\alpha = 0.9479\ h^{-1}$, $\rho_{\text{unstressed}} = 1404$ cells/μm^2. The total error with these parameters is 1122.
(B) Explant #8 [Region Ib] (initial area 0.45 mm^2): $F/k = 0.9059$, $k/b = 510\ \mu m^2/h$, $\alpha = 0.9492\ h^{-1}$, $\rho_{\text{unstressed}} = 1313$ cells/μm^2. The total error with these parameters is 820.
(C) Explant #14 [Region II] (initial area 1.12 mm^2): $F/k = 0.7948$, $k/b = 635\ \mu m^2/h$, $\alpha = 0.9410\ h^{-1}$, $\rho_{\text{unstressed}} = 1530$ cells/μm^2. The total error with these parameters is 774.
(D) Explant #17 [Region III] (initial area 2.14 mm^2): $F/k = 0.8295$, $k/b = 950\ \mu m^2/h$, $\alpha = 0.8963 h^{-1}$, $\rho_{\text{unstressed}} = 1752$ cells/μm^2. The total error with these parameters is 834.

S4 Figure. Effect of fibronectin concentration on spreading rate. Average change in area over time ($\Delta A/\Delta t$) for *Xenopus* animal cap explants that are plated on petri dishes with different concentrations of fibronectin. The error bars show the standard deviation. The spreading rate is faster for higher concentrations of fibronectin than for smaller concentrations of fibronectin. The dashed line represents the fibronectin concentration for the experiments in this paper, 25 μg/mL.

S5 Figure. Percent of Parameter Sets Accepted in ABC Rejection Algorithm Implementation.
10,000 parameter sets that resulted in total error (Eq. 13) less than or equal to a tolerance threshold of 1500 were collected for each of the 18 explants in the model building set. More than 10,000 simulations were run to obtain 10,000 accepted parameter sets, and in general, smaller explants required more simulations run than larger explants.

Supporting Videos

Time-lapse sequences of still images in the main text and supporting figures. Below contains the list of videos and which figure they animate.

S1 Video. cf_Fig_1D.avi. Accompanies Figure 1D.

S2 Video. cf_Fig_4A.avi. Accompanies Figure 4A.

S3 Video. cf_Fig_4B.avi. Accompanies Figure 4B.

S4 Video. cf_Fig_4C.avi. Accompanies Figure 4C.

S5 Video. cf_Fig_4D.avi. Accompanies Figure 4D.

S6 Video. cf_Fig_4E.avi. Accompanies Figure 4E.

S7 Video. cf_Fig_4F.avi. Accompanies Figure 4F.

S8 Video. cf_Fig_4G.avi. Accompanies Figure 4G.

S9 Video. cf_supp_Fig_3A.avi. Accompanies Supporting Information S3A Figure.

S10 Video. cf_supp_Fig_3B.avi. Accompanies Supporting Information S3B Figure.

S11 Video. cf_supp_Fig_3C.avi. Accompanies Supporting Information S3C Figure.

S12 Video. cf_supp_Fig_3D.avi. Accompanies Supporting Information S3D Figure.

Fig 1

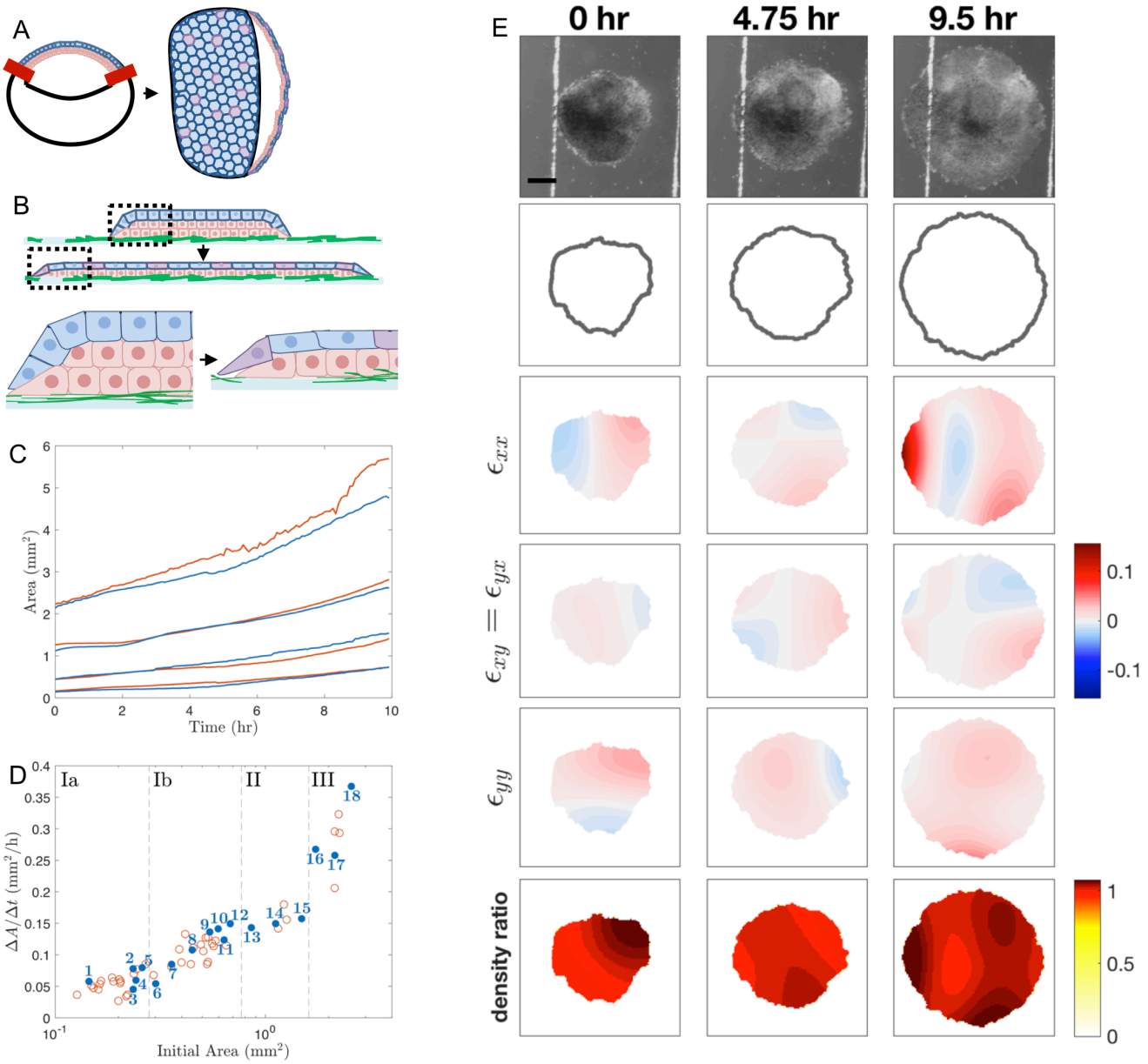

A
B
C
Area (mm²)
Time (hr)
D
Ia
Ib
II
III
ΔA/Δt (mm²/h)
Initial Area (mm²)
E
0 hr
4.75 hr
9.5 hr
ϵ_{xx}
$\epsilon_{xy} = \epsilon_{yx}$
ϵ_{yy}
density ratio
0.1
0
-0.1
1
0.5
0

Fig 2

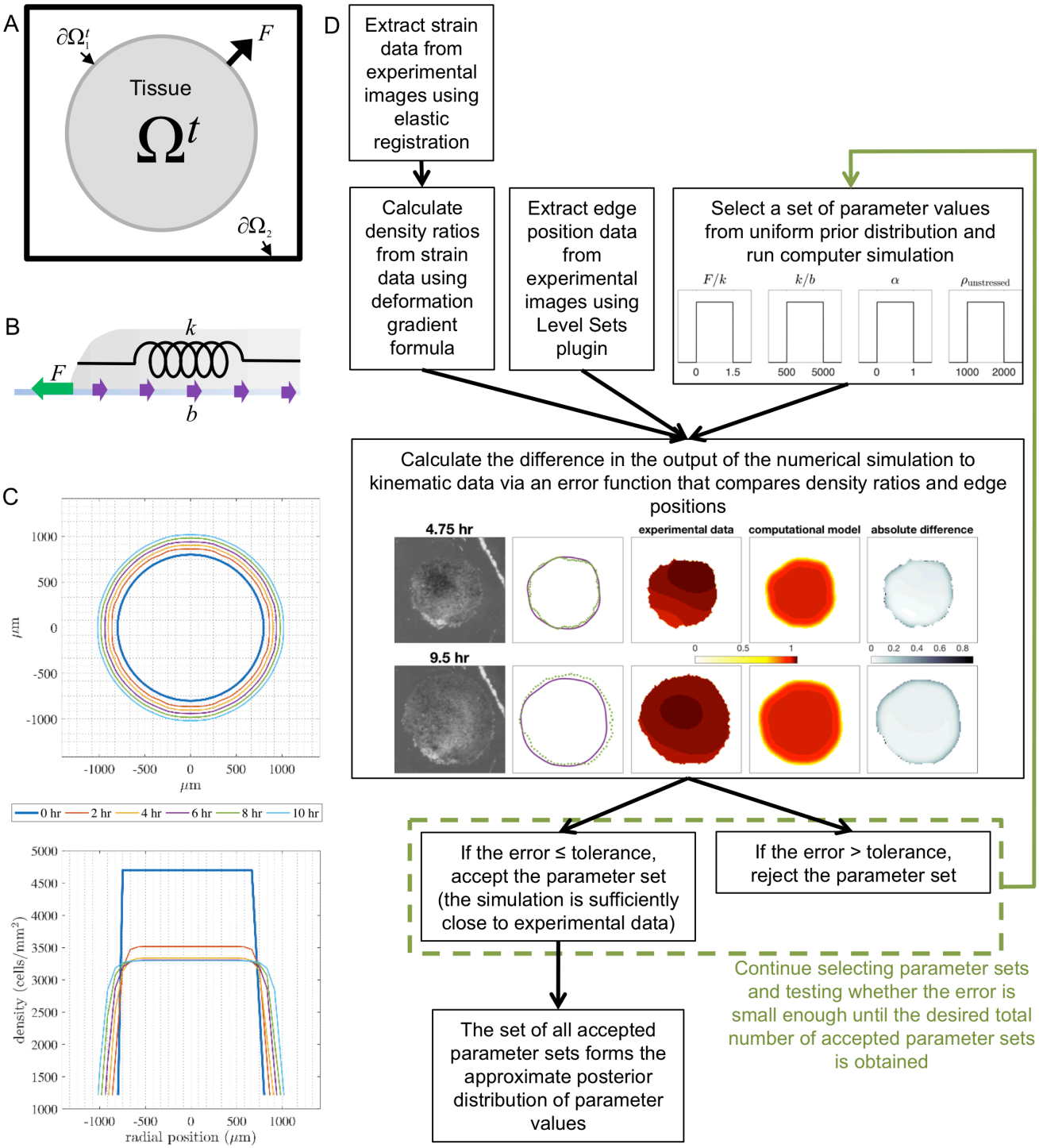

A
$\partial\Omega_1^t$
F
Tissue
Ω^t
$\partial\Omega_2$
B
k
F
b
C
1000
500
0
-500
-1000
μm
-1000 -500 0 500 1000
μm
0 hr 2 hr 4 hr 6 hr 8 hr 10 hr
5000
4500
4000
3500
3000
2500
2000
1500
1000
density (cells/mm^2)
-1000 -500 0 500 1000
radial position (μm)
D
Extract strain data from experimental images using elastic registration
Calculate density ratios from strain data using deformation gradient formula
Extract edge position data from experimental images using Level Sets plugin
Select a set of parameter values from uniform prior distribution and run computer simulation
F/k
k/b
α
$\rho_{\text{unstressed}}$
0 1.5
500 5000
0 1
1000 2000
Calculate the difference in the output of the numerical simulation to kinematic data via an error function that compares density ratios and edge positions
4.75 hr
9.5 hr
experimental data
computational model
absolute difference
0 0.5 1
0 0.2 0.4 0.6 0.8
If the error ≤ tolerance, accept the parameter set (the simulation is sufficiently close to experimental data)
If the error > tolerance, reject the parameter set
Continue selecting parameter sets and testing whether the error is small enough until the desired total number of accepted parameter sets is obtained
The set of all accepted parameter sets forms the approximate posterior distribution of parameter values

Fig 3

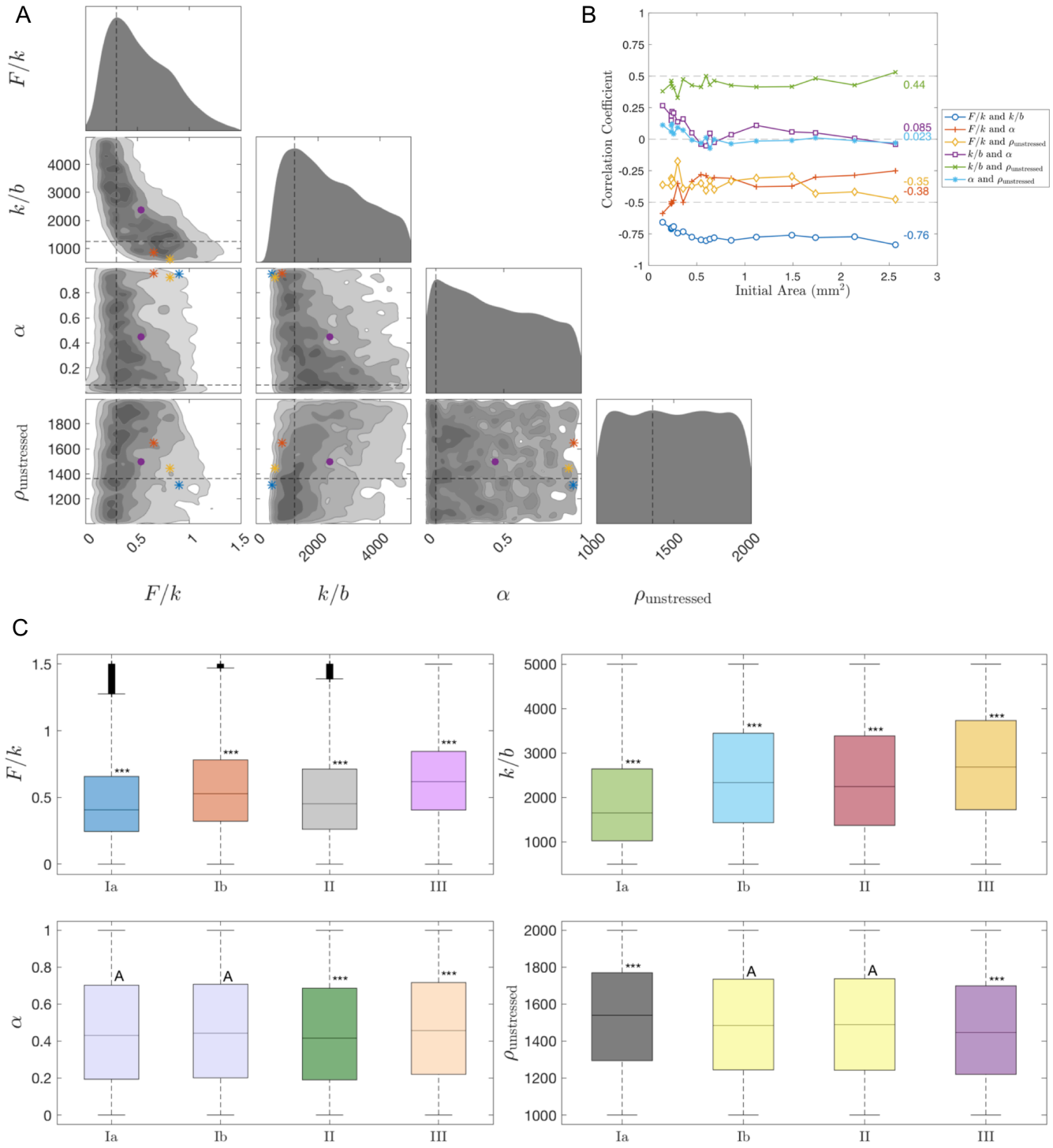
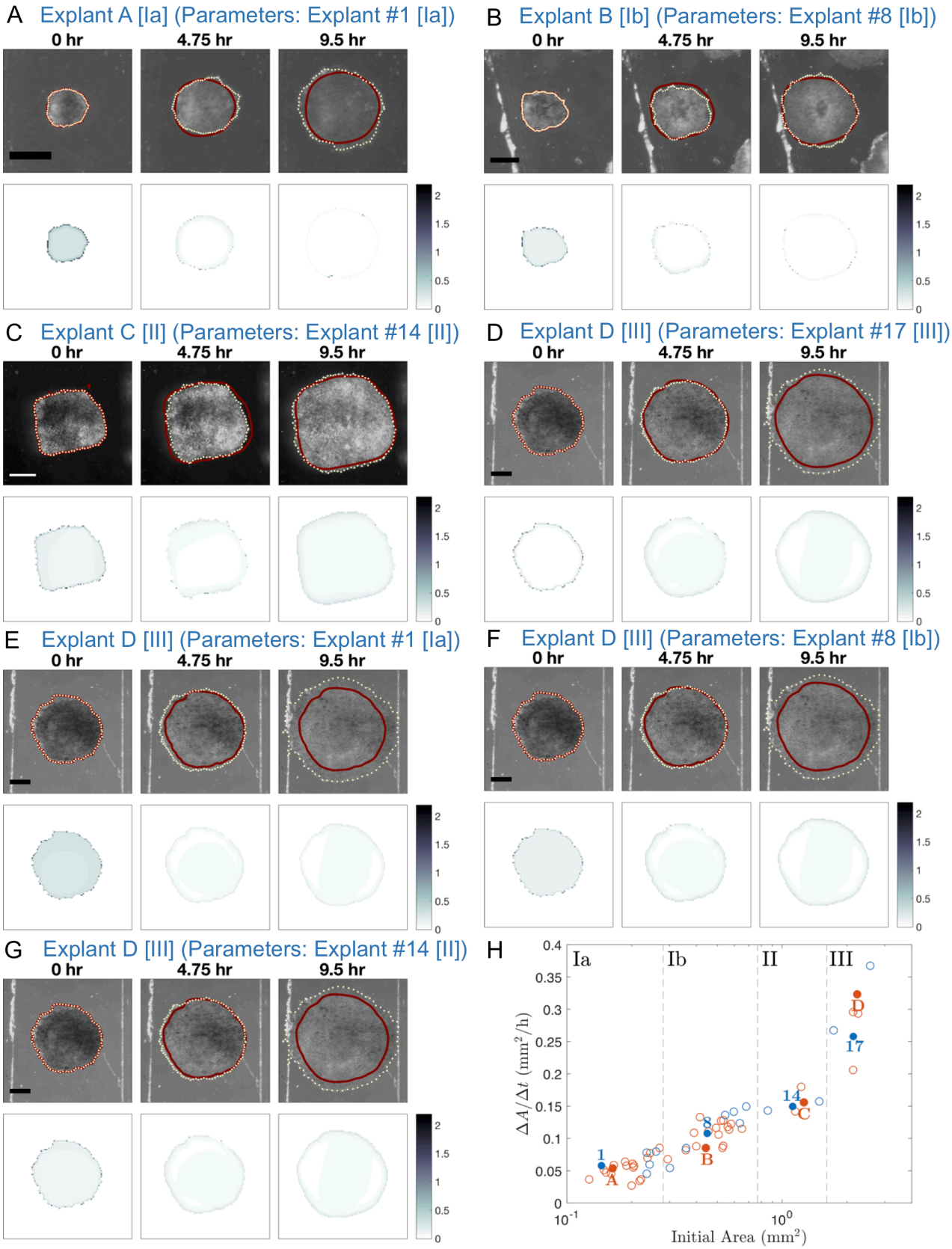
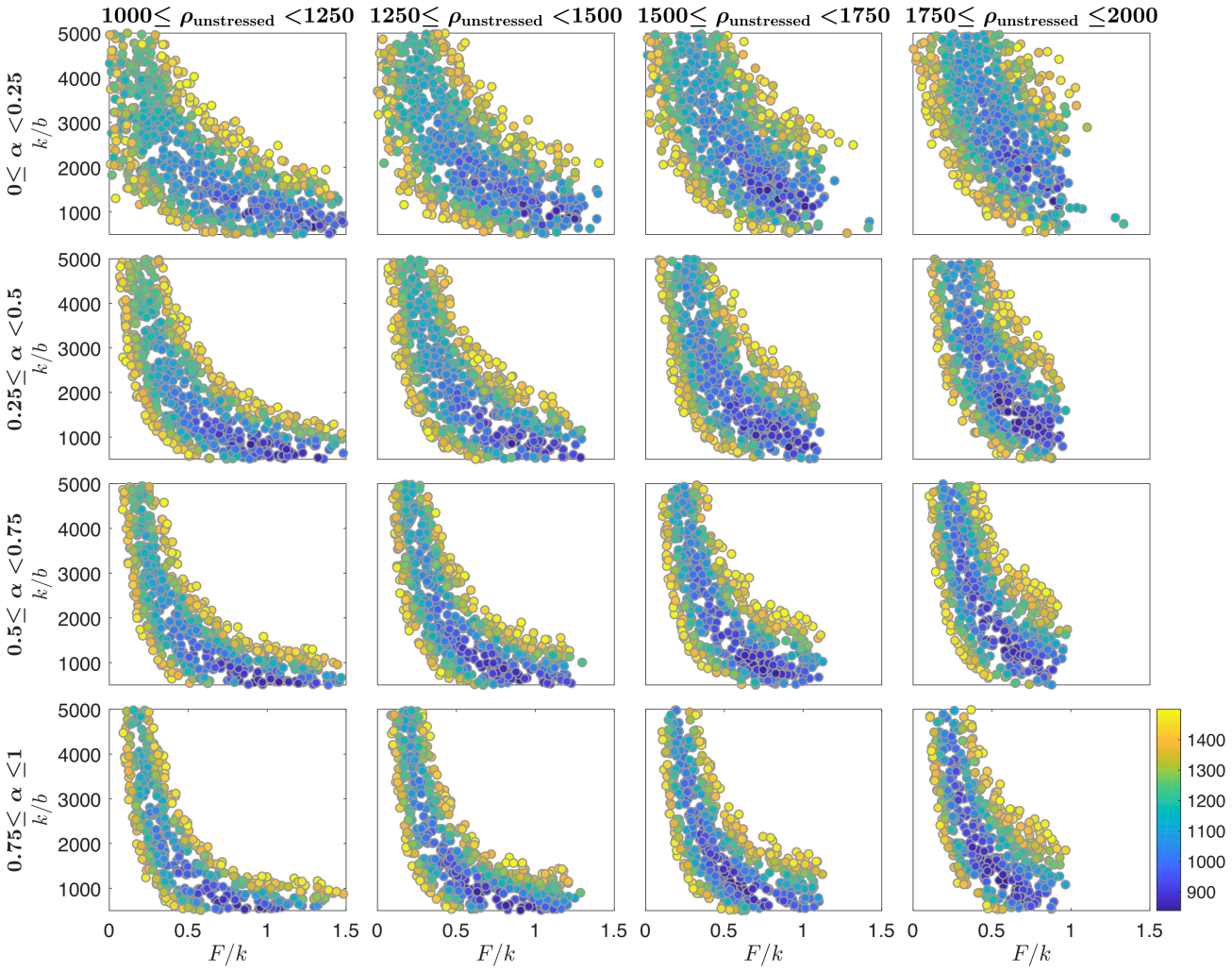

A
B
C
F/k
k/b
α
ρunstressed
Correlation Coefficient
Initial Area (mm²)
F/k and k/b
F/k and α
F/k and ρunstressed
k/b and α
k/b and ρunstressed
α and ρunstressed
0.44
0.085
0.023
-0.35
-0.38
-0.76
Ia
Ib
II
III
***
A

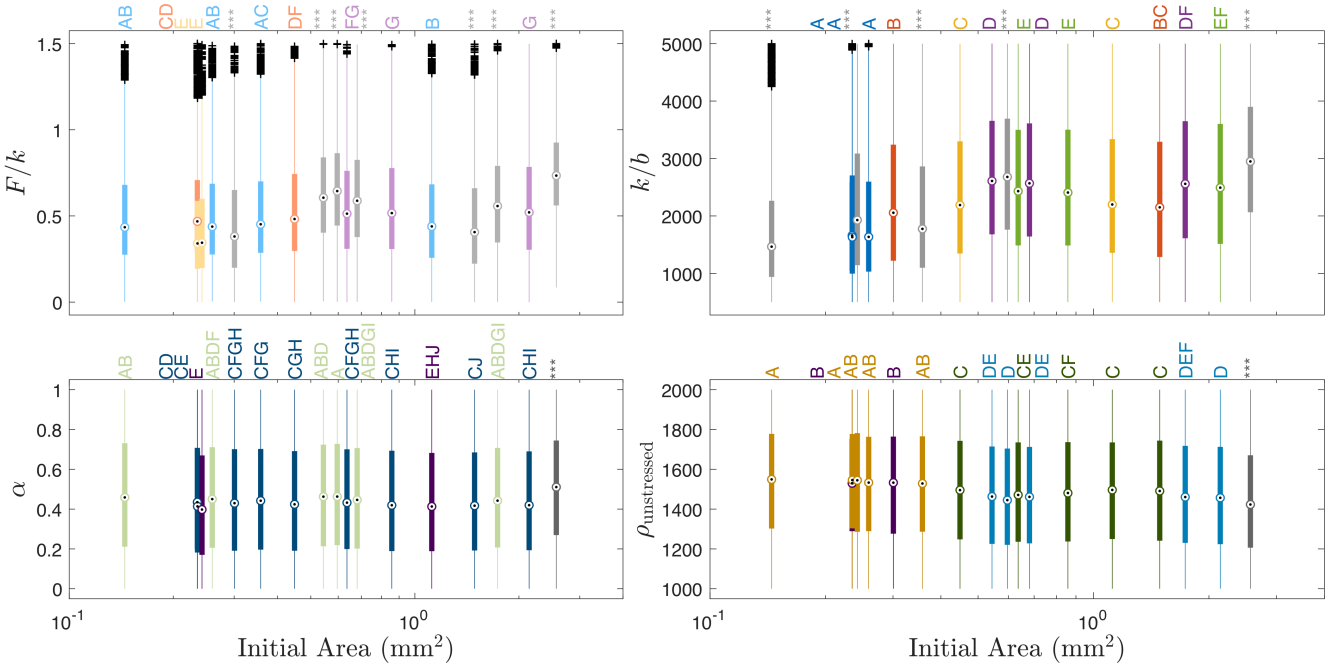
Fig 4


A Explant A [Ia] (Parameters: Explant #1 [Ia])
B Explant B [Ib] (Parameters: Explant #8 [Ib])
C Explant C [II] (Parameters: Explant #14 [II])
D Explant D [III] (Parameters: Explant #17 [III])
E Explant D [III] (Parameters: Explant #1 [Ia])
F Explant D [III] (Parameters: Explant #8 [Ib])
G Explant D [III] (Parameters: Explant #14 [II])
0 hr
4.75 hr
9.5 hr
H
Ia
Ib
II
III
$\Delta A/\Delta t$ (mm^2/h)
Initial Area (mm^2)

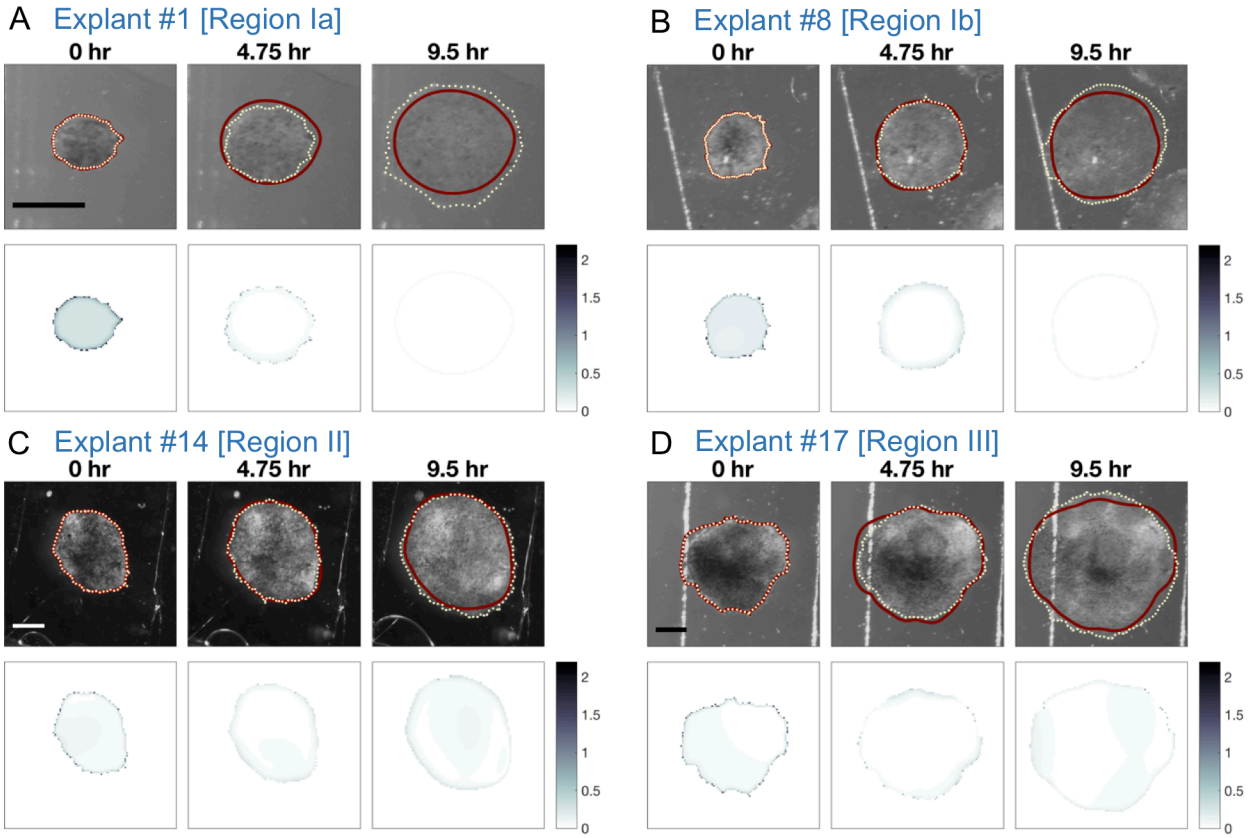
S1 Figure


1000≤ ρunstressed <1250
1250≤ ρunstressed <1500
1500≤ ρunstressed <1750
1750≤ ρunstressed ≤2000
0≤ α <0.25
0.25≤ α <0.5
0.5≤ α <0.75
0.75≤ α ≤1
k/b
F/k
5000
4000
3000
2000
1000
0
0.5
1
1.5
1400
1300
1200
1100
1000
900

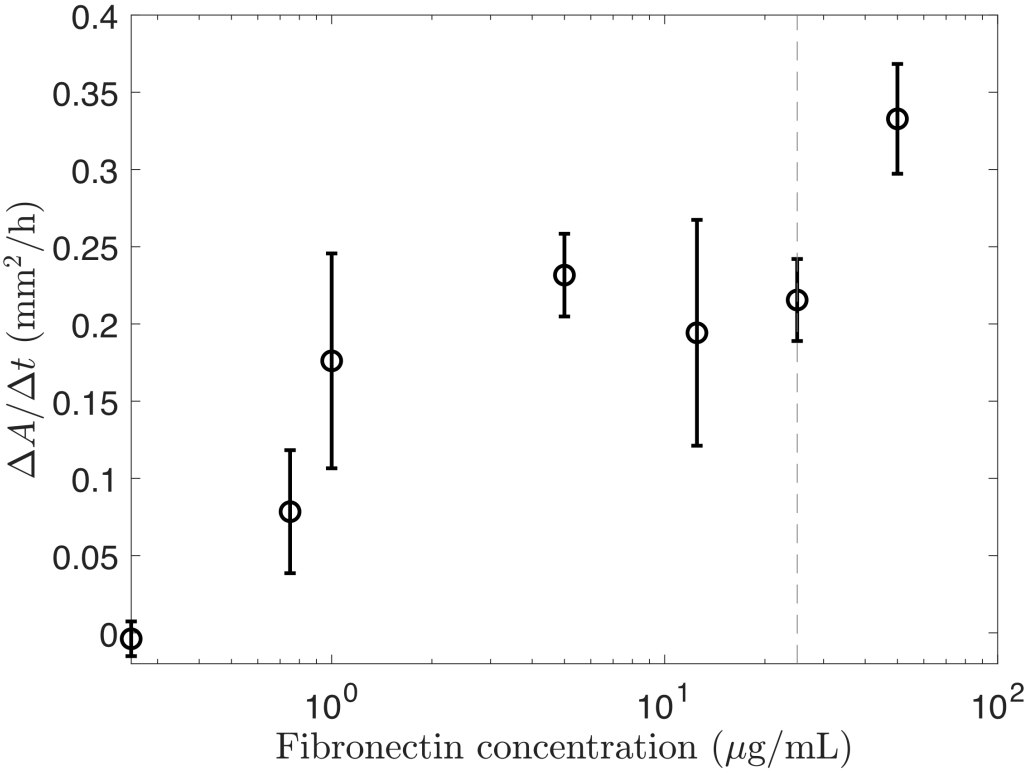
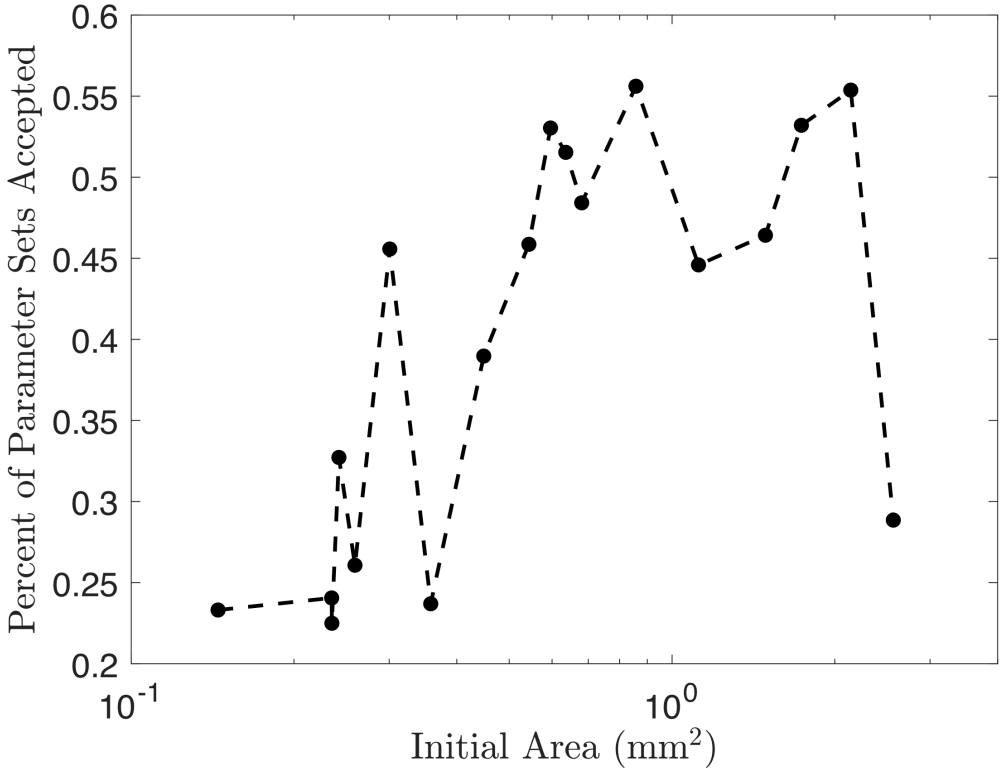
S2 Figure



S3 Figure



S4 Figure



S5 Figure