Intermittent attachments form three-dimensional cell aggregates with emergent fluid properties

Devi Prasad Panigrahi,[1] Giulia L. Celora,[2] Hugh Z. Ford,[3,4] Robert H. Insall,[5] Ramray Bhat,[6] Angelika Manhart,[7,1] and Philip Pearce[1,3,*]

[1] *Department of Mathematics, University College London*
[2] *Mathematical Institute, University of Oxford*
[3] *Institute for the Physics of Living Systems, University College London*
[4] *Laboratory for Molecular Cell Biology, University College London*
[5] *Center of Cell and Developmental Biology, University College London*
[6] *Department of Developmental Biology and Genetics, Indian Institute of Science Bangalore*
[7] *Faculty of Mathematics, University of Vienna*

Abstract. In living systems across developmental and cancer biology, populations of cells on surfaces organize themselves into aggregates that mediate function and disease. Recent experimental studies have identified that such aggregates can behave as fluid-like droplets with emergent properties such as surface tension, yet the physical origin of these properties is not well understood. Here, we develop a minimal cell-based model in which intermittent (i.e. transient) cell-cell and cell-surface attachments mimic the dynamic polarity of motile cells. We find that intermittent attachments are sufficient to generate an effective wetting-dewetting transition, which causes the formation of surface-associated three-dimensional cell aggregates. By comparing simulations with experiments, we predict that cell-surface adhesion explains morphology in aggregates of MDA-MB-231 breast cancer cells, and that chemotherapeutic resistance causes enhanced aggregate shape fluctuations through increased cell attachment strength in populations of OVCAR-3 ovarian cancer cells. Extending the model to account for biased motility, we also predict how heterogeneity in intermittent attachments governs pattern formation during the collective chemotaxis of *Dictyostelium discoideum* swarms. We show how these experimental observations in multiple biological systems can be explained by quantifiable fluid-like properties of aggregates such as wetting, surface tension, and tissue fluidity, which depend on the strength and intermittency of cell-cell and cell-surface attachments. Together, these results reveal how intermittent attachments generate cell aggregates with emergent material properties, with broad implications for development and cancer.

I. INTRODUCTION

Aggregates of motile cells perform a diverse array of physiological and pathological functions, from embryogenesis and wound healing to cancer metastasis [1–3]. Eukaryotic cell collectives can exhibit a range of complex multicellular behaviors and structures, depending on their physical interactions with the surrounding environment, from confluent tissues with long-term cell-cell adhesion [4] to motile clusters with intermittent or transient adhesion [5], which can transition from dilute populations to dense aggregates [6]. These aggregates of motile cells have been found to possess fluid-like properties that are important for numerous biological functions. For example, in intestinal epithelial development, aggregates of mesenchymal cells drive the folding of villi by forming liquid droplet-like patterns [7] (Fig. 1A(i)). Similar fluid-like properties have been observed in feeding fronts of the social amoeba *Dictyostelium discoideum*, where droplet-like patterns formed by migrating cells arise as a consequence of the emergent surface tension of the aggregate [8] (Fig. 1A(ii)). Fluid-like properties also emerge in aggregates of malignant immune cells, which exhibit an enhanced chemotactic response when migrating as a group owing to aggregate fluidity, which allows cells to exchange positions and overcome the inhibition of chemotaxis experienced by individual cells at the front of the aggregate [9]. Despite the biological relevance of the fluid-like properties of cell aggregates, the biophysical principles linking emergent aggregate properties and biological functions are not known.

In general, the formation and properties of cell aggregates are known to depend on cell-cell interactions; for example, an up-regulation of cell-cell adhesion leads to the three-dimensional (3D) aggregation of mammalian cells both *in vitro* [10] (Fig. 1A(iii)) and *in vivo* [7] (Fig. 1A(i)). Furthermore, the classification of different cell types within a developing embryo can be explained by the effective surface tension of aggregates [11]. Intermittent cell-cell adhesions have been hypothesized to result in emergent fluid-like properties such as viscosity and surface tension, which play a key role in the self-organization of cell aggregates [8, 12–14]. However, it is not known in general which physical aspects of cell-cell and cell-surface interactions control aggregate formation, three-dimensional aggregate structure, and aggregate properties.

In terms of modeling, active hydrodynamic theories have been useful in providing a macroscopic description of tissue dynamics [15, 16], but do not typically connect emergent phenomena directly to cell behaviors. By con-

* philip.pearce@ucl.ac.uk

trast, vertex models can explain the emergence of fluid-like properties of epithelial tissues owing to mechanical interactions between confluent cells [17–20]. For example, such models have been applied to understand the role of sub-cellular structures on the emergent material properties of embryonic tissues [21]. However, since vertex models are generally applicable to confluent monolayers, they have not yet been applied to capture the emergence of three-dimensional aggregates of motile cells from dilute populations. An alternative modeling approach is to represent cells as discrete particles with interaction forces between them. This approach has been applied to capture the emergence and dynamics of three-dimensional surface-associated bacterial aggregates through growth and physical cell-cell interactions such as steric repulsion, and attraction mediated by extracellular matrix proteins [22, 23]. More widely, particle-based models have been used to predict specific physical properties of dense aggregates, including the size and coalescence of bacterial aggregates, caused by retractable appendages such as type IV pili [13, 14]; coalescence and solid-to-fluid transitions in stem cell aggregates with intermittent attractions [24]; surface tension of surface-associated amoeboid cell aggregates with permanent cell-cell adhesions [25]; and the organization of two-dimensional cell aggregates as a function of contact-inhibition of locomotion [26]. However, it is not well understood how cell interactions mediate the transition from dilute surface-associated populations to dense aggregates with emergent properties. We therefore lack a multiscale physical framework for the self-organization of eukaryotic cells into three-dimensional structures with fluid-like material properties.

Here, to bridge these gaps in biological and physical understanding, we develop a cell-based model with intermittent or transient cell-cell interactions as a coarse-grained representation of the dynamics of the actin cytoskeleton. We hypothesize that the cytoskeletal activity of the cell, along with cell-cell adhesion, can give rise to intermittent attractive forces between cells, which we model as pairwise active reciprocal interactions. We show that differential adhesion to the surface or substrate, along with intermittency in attractive forces, can explain the dewetting of cell layers to form three-dimensional aggregates. Despite the simplicity of our model, we show that it can explain several key features observed in experiments; our hypotheses can be verified in future experiments that measure how different dynamics of individual cells (e.g. between different cell lines or under different conditions) affect emergent properties of aggregates.

II. CELL-BASED MODEL WITH INTERMITTENT ATTACHMENTS

Motivated by recent experiments showing fluid-like cell aggregates in various living systems (Fig. 1A), we aimed to understand how eukaryotic cells on surfaces or substrates organize themselves into aggregates with macroscopic or emergent properties. A common feature of eukaryotic cell motility is the presence of physical interactions between cells and their surrounding environment through actin-rich protrusions on the cell membrane, such as lamellipodia or pseudopodia, which are formed along the direction of the cell's polarity [27]. These have been observed in a diverse range of cells, including cells within developing embryos [28], neutrophils [29], fibroblasts [30], and metastatic cancer cells [31]. During motility, these protrusions form temporary attachments to features of the external environment, such as the membrane of a nearby cell or the solid substrate over which a cell is migrating [28, 32]. Retraction of an attached protrusion causes a local and transient generation of force, which is transmitted to the cell body through long bundles of actin filaments, effectively pulling the cell in the direction of the protrusion [33]. The polarity of motile cells is dynamic, so that a protrusion can detach and retract, and a new one may form and extend in a different direction [34]. Physically, these features of eukaryotic cell motility can be considered as effectively causing cells to intermittently attach and pull themselves towards external surfaces or other cells, which feel an equal and opposite force.

To capture these features of cell motility and physical cell interactions in both dilute and dense populations of cells, we built a minimal 3D cell-based model with intermittent or transient cell-cell and cell-surface attachments. In our model, cells are represented as rigid spheres which cannot overlap. We model this using a soft repulsive Weeks-Chandler-Andersen (WCA) potential between any two cells, so that the ith cell experiences a force

$$\mathbf{F}_{ij}^{\text{WCA}} = \left[2\left(\frac{\sigma}{r_{ij}}\right)^{12} - \left(\frac{\sigma}{r_{ij}}\right)^{6}\right]\frac{24\varepsilon\hat{\mathbf{r}}_{ij}}{r_{ij}},\ r_{ij} \leq 2^{\frac{1}{6}}\sigma \quad (1)$$

because of the presence of the jth cell. In Eq. (1), ε denotes the strength of the repulsive force, $\mathbf{r}_{ij}$ is the inter-sphere distance, $r_{ij} = |\mathbf{r}_{ij}|$ denotes the separation distance, $\hat{\mathbf{r}}_{ij}$ denotes the unit vector along the position vector between the two cells, and σ is the length scale of repulsion (corresponding to the volume exclusion experienced by the cells). When the distance between the centroids of two cells becomes less than $2^{1/6}\sigma$, they are pushed apart (Fig. 1B) to avoid overlapping. For $r_{ij} > 2^{1/6}\sigma$, the repulsive force is zero.

In the model, we capture intermittent attachments by introducing transient springs that form between spheres and their neighbors, and pull them towards each other (Fig. 1C; see Methods for more details). Briefly, at all times, each cell is assumed to be polarized along a certain direction towards a neighboring cell within a certain distance; a temporary attachment connects the two cells. The attachment is represented as a Hookean spring with a natural length that is shorter than the current distance between the two cells. The spring remains present for a

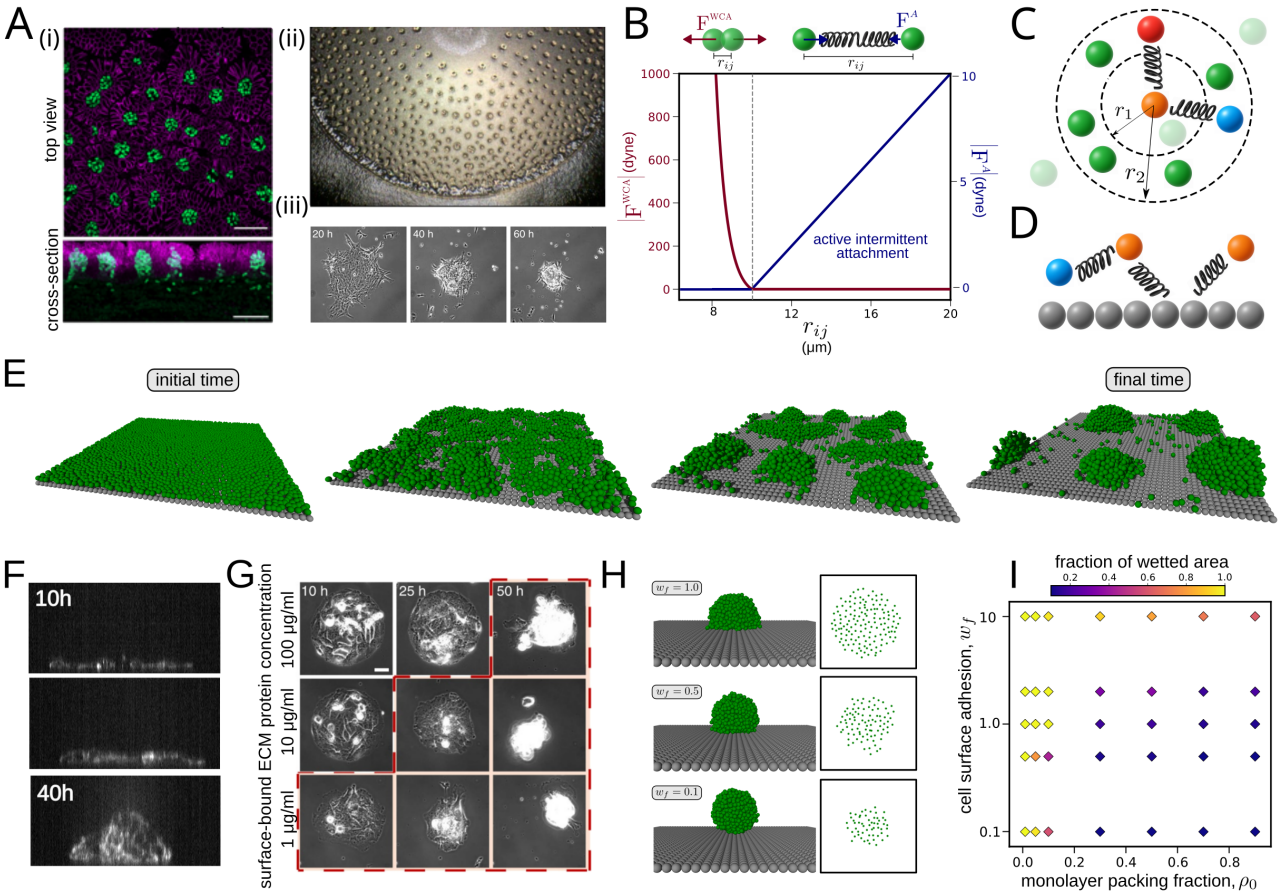

FIG. 1. **3D cell-based model with intermittent attachments captures wetting and dewetting of cell aggregates on surfaces.** (A) Experiments showing fluid-like cell aggregates. (i) droplet-like patterns formed in the intestine of a developing embryo, where the patterns govern the folding of villi, obtained from [7] (licensed under CC BY 4.0), (ii) the pattern formed by swarms of *Dictyostelium discoideum* migrating over a bacterial lawn, obtained from [8] (licensed under CC BY 4.0), (iii) droplet-like aggregate formed by breast cancer cells (from [10], reproduced with permission from SNCSC). (B) Magnitude of the force due to WCA repulsion and intermittent active attachment between two attached cells. (C) Schematic of the cell-cell attachments showing the formation of active attachments. Colors are for labeling only – all cells behave the same. The orange cell can form an attachment to any other cell in the region between the two dashed circles with radii r_1 and r_2, e.g. the blue cell. It cannot attach to the faded green cells, and all other cells in the same region can also attach to the orange cell, e.g. the red colored cell forms such an attachment. See Methods for a detailed explanation, and Movie S1 for a visualization of the intermittent attachments. (D) Schematic depicting simultaneous cell-cell and cell-surface attachments. Grey spheres represent the surface and are fixed. The orange cells form attachments to the surface. The blue cell forms an attachment to the left orange cell. (E) Snapshots of a simulation showing that a monolayer of cells can spontaneously de-wet a surface, forming clusters that are nearly hemispherical in shape, closely resembling liquid droplets. The cells are represented by green spheres, and the surface by gray spheres. (F) Experimental snapshots showing the dewetting of an aggregate of MDA-MB-231 breast cancer cells (from [10], reproduced with permission from SNCSC). (G) In the experiments, a decrease in concentration of a surface-bound ECM protein (from top row to bottom row; left to right is increasing time) leads to a decrease in wetted area (from [10], reproduced with permission from SNCSC). (H) Simulations showing the 3D cell aggregate (left), and the corresponding contact area (right) for different values of cell-surface adhesion strength w_f in Eq. (3). The contact areas in H and the right-most column of G, representing the cell aggregate at steady state, appear visually similar because the microscopy imaging predominantly captures the cell-substrate interface. (I) Variation in the fraction of the surface area wetted by the cells at steady state as a function of differential cell-substrate adhesion, and packing fraction of the cell monolayer. The colorbar represents the ratio of the surface area wetted by the cells at steady state relative to the initial condition, which is fully wetted (see left-most panel in E).

certain duration of time, sampled from a Gaussian distribution with mean T_a (corresponding to the timescale for persistence of cell polarity) and variance T_v, and then disappears. The cell then instantaneously re-polarizes, and a new spring is formed with a neighbor (this may be the same neighbor as previously or a different neighbor). Therefore, at any moment in time, each cell is polarized (i.e. forms an attachment) towards exactly one neighbor-

ing cell. However, a cell might also be attached to other cells through their polarities (Fig. 1C, Movie S1). Mathematically, these interactions are captured in a transient interaction force between cells and their neighboring cells

$$\mathbf{F}_i^A = \sum_{j \in P_i} F_a(r_1 - r_{ij})\hat{\mathbf{r}}_{ij}, \quad r_{ij} \geq r_1 \tag{2}$$

where P_i denotes the set of interacting cell partners of the ith cell, F_a denotes the strength of cell-cell attachment, and r_1 is the minimum distance between two cells at which an attachment can form (Fig. 1C). For simplicity of modeling and implementation, the surface or substrate is represented as a layer of rigid immovable spheres with the same size as the cells, to which cells can attach in the same way as other cells (Fig. 1D). To vary cell-cell and cell-surface attachments independently, the model includes a parameter w_f that multiplies the strength of cell-surface attachments relative to cell-cell attachments to give

$$\mathbf{F}_i^s = \sum_{j \in S_i} w_f F_a(r_1 - r_{ij})\hat{\mathbf{r}}_{ij}, \quad r_{ij} \geq r_1 \tag{3}$$

where S_i denotes the set of interacting surface partners of the ith cell (the size of the set S_i is at most one).

Cell motility occurs at very small length scales, where the inertia of individual cells can be neglected in comparison to viscous effects. Thus, our model captures the motion of cells through the overdamped equation of motion

$$\frac{d\mathbf{X}_i}{dt} = \mu\left(\mathbf{F}_i^{\text{WCA}} + \mathbf{F}_i^A + \mathbf{F}_i^s\right). \tag{4}$$

Cells in dense populations may form multiple simultaneous attachments to other cells [35]. However, it is known that cell motility is driven by the presence of an imbalance in forces along a particular direction arising from a stronger attachment in that direction [36], which we model explicitly. We assume that the effect of other attachments impedes the motion of the cell, and consider this effect to be implicit in the isotropic viscous response of the cell to the surrounding fluid media through the effective mobility parameter μ, which also captures the effect of the external fluid, and any extracellular matrix; in all simulations μ is assumed to be spatially uniform and constant for simplicity. Eq. (4) does not contain a thermal noise term, because eukaryotic cells are too large to feel significant thermal diffusion in comparison to their active motility – stochasticity in the model arises only through the random choice of other cells when a cell forms a new attachment.

To simulate populations of cells with intermittent attachments, we integrate Eqs. (1)-(4) numerically using LAMMPS [37]. A typical simulation consists of approximately 1000 cells with periodic boundary conditions in all directions unless they are bound by a surface. Unless specified otherwise, the mean attachment time T_a is assumed to be equal for cell-cell and cell-surface attachments, and the default values of the various parameters in the model are chosen to be in line with experimental data (see Methods and Supporting Information). All quantitative data obtained from the simulations were averaged over a sufficiently large ensemble to eliminate artifacts arising from stochasticity. In general, we define a simulation as having reached a steady state when the output property of interest is roughly constant. The initial conditions were chosen depending on the system being studied (see Supporting Information).

III. RESULTS AND DISCUSSION

A. Intermittent cell-cell and cell-surface attachments control dewetting of 3D cell aggregates

To establish the role that intermittent cell-cell and cell-surface attachments play in self-organisation, we performed simulations of our model Eqs. (1)-(4). We found that, for certain choices of model parameters, a monolayer of surface-associated cells spontaneously self-organizes into three-dimensional (3D) aggregates that qualitatively resemble liquid droplets (Fig. 1E, Movie S2) – we refer to this as "dewetting" the surface. We quantified this dewetting by measuring the fraction of surface area in contact with the cells (see Supporting Information), which decreases as the dewetted aggregates form, and plateaus at steady state (Fig. S1A). To investigate the role of intermittency in the dewetting transition, we varied the average duration T_a of cell attachments. When T_a is very small, dewetting does not occur, because the cells need to apply a force for a sufficiently long duration of time to overcome the repulsive energy barrier required for cell rearrangements and transitioning into 3D aggregates (Fig. S1B). For very large T_a, cells are unable to rearrange because cell-cell attachments persist for too long, again precluding the formation of 3D aggregates over the timescale of our simulations, even if cell-surface attachments are intermittent (Fig. S1C,D). These results suggest that intermittent attachments allow cells to move on surfaces and form associations with other cells, causing the transition from dilute populations to dense aggregates, and to rearrange within dense aggregates, causing the formation of 3D structures.

Next, we used our model to interpret recent experimental results [10] on dewetting of aggregates of MDA-MB-231 breast cancer cells, in which extensive cell rearrangements occur in 2D and 3D (Fig. 1F,G). In these experiments, it was shown that a cell monolayer can dewet a surface upon increase in cell-cell adhesion due to induction of E-Cadherin (Fig. 1F), with qualitative similarities to our results (Fig 1E). Furthermore, it was found that a decrease in the concentration of a surface-bound collagen, which is an extracellular matrix (ECM) protein, leads to a decrease in wettability (Fig. 1G). We modeled the change in collagen concentration as an effective

decrease in cell-surface attachment strength in comparison to that of cell-cell attachments by modifying the parameter w_f in Eq. (3). We found that a decrease in cell-surface attachment strength via w_f causes a reduced contact area of the cells with the surface at steady state, in line with the experimental measurements (Fig 1H). To further characterize this effect in our model, we generated a regime diagram for the fraction of wetted area in steady state as a function of the initial packing fraction of the cell monolayer ρ_0 and the differential surface attachment strength w_f (Fig. 1I). We found that an increase in w_f leads to a decrease in the fraction of the wetted area, irrespective of the number of cells in the monolayer (represented by ρ_0). This suggests that the relative strength of intermittent cell-cell and cell-surface attachments can explain the wettability of cell populations and the ability of aggregates to transition into 3D structures.

B. Intermittent cell-cell and cell-surface attachments determine shape fluctuations of 3D cell aggregates

In recent experiments comparing aggregates of ovarian cancer OVCAR-3 cells to aggregates of chemotherapeutic drug-resistant OVCAR-3 cells, it has been observed that the resistant cells spread more effectively on a substrate compared to the drug-sensitive cells [38]. Drug-resistant cells were also observed to exhibit increased single-cell motility, measured via trans-well migration assay [39], with implications for invasiveness *in vivo*. Furthermore, aggregates of drug-resistant cells were measured to be much less circular and to exhibit more pronounced shape fluctuations (Fig 2A). At the cell level, drug-resistant cells were found to exhibit increased expression of both E-Cadherin and Fibronectin (Fig. 2B), which mediate cell-cell and cell-substrate adhesion, respectively, indicating a combined increase in both cell-cell and cell-substrate adhesion [40, 41]. We aimed to explore a potential connection between these cell- and aggregate-level observations.

To investigate the effect of increasing both cell-cell and cell-surface interactions in our model, we performed simulations for different values of F_a in Eq. (2), which determines the strength of both types of attachment (we assumed that each attachment has the same strength, $w_f = 1$). We explored whether this was sufficient to recapitulate experimental observations. First, to probe single-cell dynamics, we assessed the motility of isolated cells, finding that increasing F_a enhances motility (Fig. S2A), in line with experiments [38] (Fig. S2B). To probe the collective behavior, we then simulated 3D aggregates. As before, we assume the aggregate is at steady state when the contact area of the aggregate with the surface remains roughly constant with time. We found that an increase in F_a leads to a monotonic increase in the fraction of wetted area at steady state (Fig. 2C, Fig. S2C). To analyze shape fluctuations in our simulations, we extracted the convex hull of cell centroids within an aggregate, when projected onto a plane parallel to the surface, and quantified its deviation from a circle (see Supporting Information). We defined a cell as belonging to the aggregate if they were not in the wetting layer near the surface (see Supporting Information). We found that for higher values of F_a, the aggregates contain fewer cells due to enhanced wetting, and their shape both fluctuates (Fig. 2D) and deviates (Fig. 2E) from a circle by a greater amount. These fluctuations lead to the extension of multicellular protrusions along the aggregate surface (Movie S3), in agreement with the experiments (Fig. 2A). These shape fluctuations are driven by the increase in cell attachment strength – for example, increasing the cell-surface differential adhesion parameter w_f increases wetting without strongly affecting the magnitude of shape fluctuations (Fig. S2D). These results suggest that, although chemotherapeutic resistance of cancer cells might be expected to induce a wide range of changes in cell behavior, changes to cell attachments are sufficient to explain observed changes to overall aggregate morphology. In general, our simulations predict that the overall strength of intermittent cell-cell and cell-surface attachments increases shape fluctuations and wetting of surface-associated cell aggregates.

These cell- and aggregate-level observations can be understood by considering the movement of a cell induced by a given attachment. In our model, an increase in F_a causes cells to travel further before an attachment breaks (after a time T_a), provided the attaching spring does not eventually reach its natural length. This enhanced persistence of motion can be expected to increase individual motility, in line with previous work [42]. At the aggregate level, enhanced persistence of cell motion causes cells near the aggregate boundary that are polarized towards the surface in a direction external to the aggregate to travel further before either returning to the aggregate or escaping entirely. This suggests increased persistence enhances both wetting and shape fluctuations. To confirm this, we fixed F_a and varied the attachment time T_a, which also increases the distance traveled during cell attachments. We found that an increase in T_a also causes increased wetting and shape fluctuations (Fig. S2E,F), confirming that enhanced persistence of cell motion causes enhanced shape fluctuations of aggregates. Taken together, these results suggest that the strength and intermittency of cell-cell and cell-surface attachments govern biologically relevant properties of cell aggregates through the active motion of individual cells.

C. Bias in intermittent attachments generates collective migration of 3D cell aggregates

Collective cell migration is a fundamental biological phenomenon with physiological relevance in disease [43] and development [44]. Recently, aggregates or swarms of *Dictyostelium discoideum* cells have been found to col-

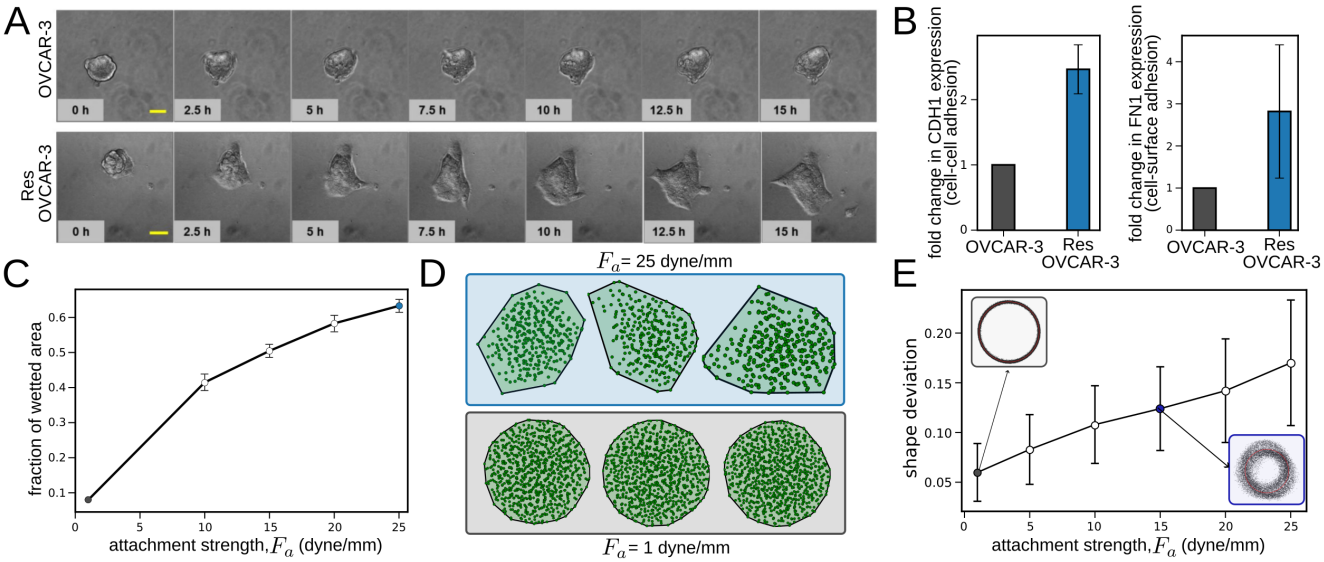

FIG. 2. **Intermittent attachments mediate wetted area and shape fluctuations of cell aggregates on surfaces.** (A) Time-lapse imaging of wetting of an aggregate of OVCAR-3 ovarian cancer cells (top), and drug-resistant OVCAR-3 ovarian cancer cells (bottom), on a substrate coated with collagen-I. Comparison of the top and bottom rows reveals that aggregates of drug-resistant OVCAR-3 cancer cells exhibit greater surface wettability and shape fluctuations (obtained from [38], licensed under CC BY-NC-ND 4.0). (B) Experimental measurements of the expression levels of E-Cadherin (left) and Fibronectin (right), which govern, respectively, cell-cell and cell-surface adhesion, reveal both proteins are upregulated in drug-resistant cancer cells. Data have been obtained from [38], licensed under CC BY-NC-ND 4.0. (C) Variation in the fraction of the surface area wetted at steady state with the attachment strength F_a when $w_f = 1$. An increase in F_a leads to a simultaneous increase in both cell-cell and cell-surface attachments. (D) Snapshots of the top view of a cell aggregate at steady state (i.e. when aggregate-surface contact area remains roughly constant with time), for two different values of F_a. The black colored line denotes the approximate boundary of the aggregate, included to highlight the shape fluctuations. Cells outside the aggregate are not plotted. A 3D rendering of these simulations is shown in Fig. S2C. (E) Variation in shape deviation with attachment strength F_a for the simulations corresponding to C. The insets show the points along the boundary of the aggregate at different instances of time, after it has attained a steady state. A complete list of model parameters other than the ones mentioned here can be found in the Supporting Information (Table S1).

lectively migrate towards nutrients because of chemotactic bias, while displaying extensive cell rearrangements and pattern formation caused by periodic splitting of the swarm front as it migrates [8] (Figs. 1A(ii) and 3A). Similar cell rearrangements have been observed in the collective chemotaxis of malignant immune cells [9]. Motivated by these experiments, we aimed to explore whether intermittent attachments are sufficient to explain collective chemotaxis of cell aggregates, pattern formation caused by aggregate splitting, and cell rearrangements within aggregates during migration.

First, we explored whether biased intermittent attachments are sufficient to explain collective migration of aggregates. We performed simulations of aggregates with bias in the direction in which cells' intermittent attachments form, determined by a self-generated chemoattractant gradient (see Supporting Information, Fig. S5). A common form of robust and long-range chemotaxis involves the localized consumption of chemoattractant by cells, giving rise to a self-generated gradient in chemoattractant concentration which replenishes as cells move towards the chemoattractant [45]. As a simple representation of this process, we considered a one-dimensional field of chemoattractant that can freely diffuse in space, and is consumed locally by a migrating front of cells (see Supporting Information). To represent bias in the model, we allowed for preferential attachment to neighbors in the direction of increasing chemoattractant, with a probability that increases with the strength of the gradient in receptor occupancy (with a plateau at high gradients; see Supporting Information). We found that this was sufficient for long-distance migration of aggregates, which follow a chemoattractant concentration gradient that travels with the aggregate (Fig. 3B). During migration, the bias in the direction of attachment is non-uniform within the aggregate – bias is higher towards the leading edge of the aggregate, where cells experience a higher gradient in chemoattractant concentration because of chemoattractant consumption within the aggregate (Fig. 3B). A higher bias in the leading edge of the aggregate might be expected to cause the aggregate to spread out over time, with leading cells traveling faster. Despite this, we find that for certain values of the parameters, the aggregates maintain an approximately steady shape and speed during migration (Fig. S3A); by contrast, under conditions that favor wetting, aggregates do not remain

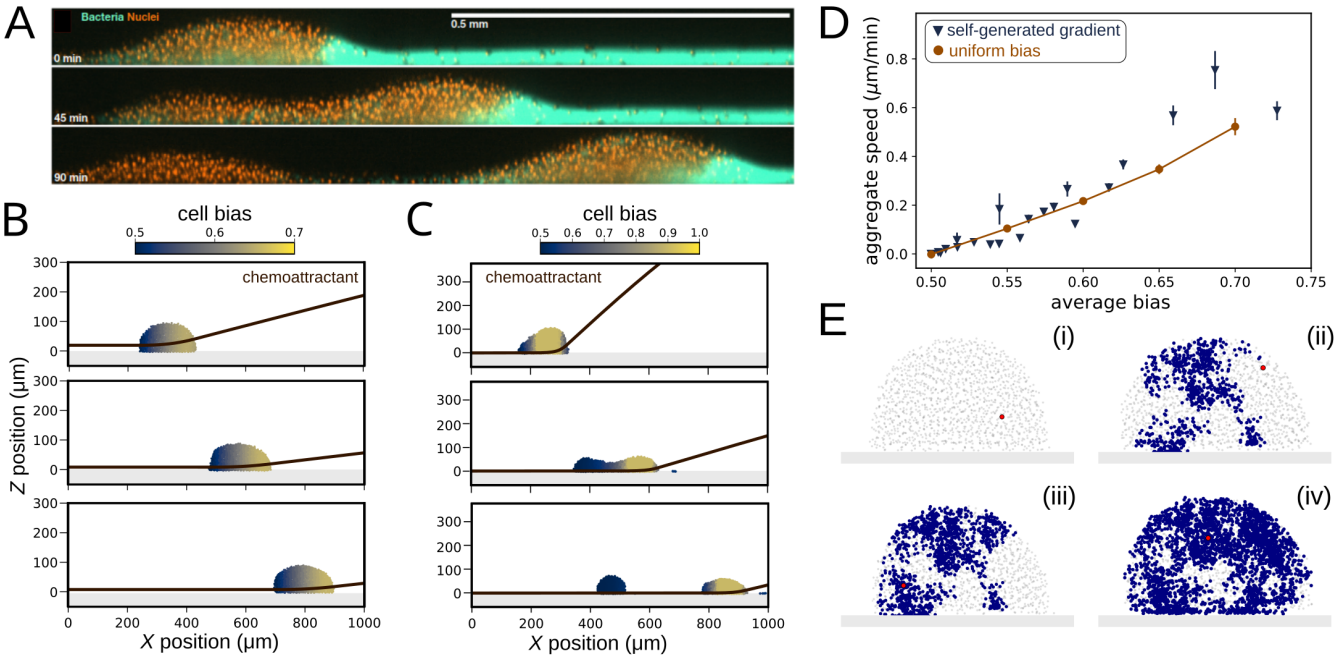

FIG. 3. **Intermittent attachments with bias cause migration of cell aggregates with emergent surface tension and fluidity.** (A) Snapshots from experiments on aggregates of *Dictyostelium discoideum* undergoing chemotaxis to bacteria (obtained from [8], licensed under CC BY 4.0). The orange fluorescent colored dots denote the position of the cell nuclei, and the green fluorescent color denotes the bacteria. (B) Migration of a cell aggregate with surface tension owing to self-generated chemotaxis and biased intermittent attachments. The color denotes bias experienced by single cells, and time increases from top to bottom. Here, the position of the cells is overlaid with the chemoattractant concentration profile, which is appropriately scaled, in order to visualize the collective chemotaxis. The surface is illustrated by the gray shaded area, and bias affects both cell-cell and cell-surface interactions. The aggregate stays together despite the spatial variation in bias, indicating an emergent surface tension. By contrast, under conditions that favor wetting, the aggregate spreads out (Fig. S3B). Further details on the boundary conditions for the chemoattractant concentration field, model parameters (Table S1), and mathematical implementation are in the Supporting Information. (C) Migration and splitting of an aggregate of cells because of emergent surface tension from intermittent attachments; splitting has qualitative similarities to experiments in panel (A). In the model, the rate of degradation of the chemoattractant is higher in C as compared to B, which causes splitting. Other factors that cause splitting include increased aggregate size and chemotactic sensitivity; see Fig. S3. (D) Comparison of the migration speed of aggregates in self-generated chemotaxis (similar to panels B and C), and simulations where all the cells in the aggregate are fixed to have the same value of bias (i.e. uniform bias model). The migration speeds of the aggregates in self-generated chemotaxis are similar to those of uniformly biased aggregates with the same average bias. (E) Intermittent attachments generate an emergent tissue fluidity in migrating aggregates. Footprint of a single particle within an aggregate (time increasing from (i) - (iv)), for the simulation corresponding to (B). The red colored dot denotes the current position of the cell, and the blue color denotes the positions occupied by the cell in all preceding instances of time.

coherent during migration (Fig. S3B). Furthermore, we find that the migration speed of aggregates is generally similar to the speed at which they would migrate if the attachment bias of all cells were imposed to be the same as the average self-generated bias (see Fig. 3D). In line with experiments and continuum modeling [8], this suggests that migrating aggregates are held together by an emergent surface tension induced by intermittent cell-cell attachments.

Second, to investigate how fluid-like properties emerge and mediate pattern formation in migrating aggregates, we aimed to reproduce previously observed splitting of swarms during migration [8]. We found that an enhanced heterogeneity of bias within a migrating aggregate can lead to splitting, where a small aggregate of non-biased cells is left behind an aggregate that continues to migrate at reduced size (Fig. 3C). For a given parameter set, there exists a critical size of the aggregate beyond which it splits (Fig. S3C). This can explain the repeated splitting events seen in experiments and continuum modeling of active droplets [8], where cell proliferation gradually increases the size of the aggregate to its critical size after each splitting event. An enhanced spatial heterogeneity in bias can also be induced by increasing the rate of chemoattractant degradation or the chemotactic sensitivity of cells (Figs. S3D,E), showing how the heterogeneity in nematic alignment that drives the splitting of active droplets [8] emerges from changes in the bias of intermittent attachments at the cell scale. Together, these results show that emergent aggregate properties arising

from intermittent attachments between cells can explain pattern formation caused by splitting of migrating aggregates during collective chemotaxis.

Finally, we investigated whether intermittent attachments are sufficient to explain cell rearrangements during aggregate migration. Experiments reveal that the cells move around within migrating aggregates over timescales much faster than cell thermal diffusivity [8, 9]. In general, this process is observed across many tissue types and is referred to as "tissue fluidity" [46, 47]. Biologically, this emergent random motion enables homogeneity in the properties of cells within migrating aggregates favoring access to nutrients and chemoattractants, which are mainly localized near the leading edge of the aggregate – we do not separate nutrients and chemoattractants in our modeling, but their concentrations are often correlated [8]. In *D. discoideum*, this fluidity prevents the occurrence of starvation-induced differentiation within the population during migration [48], leading to a migrating aggregate in which the cells are synchronized at the same point in their differentiation program.

This system is therefore fundamentally different from other examples of collective cell migration driven by leader-follower dynamics. In aggregates of cancerous immune cells, fluidity contributes to the efficiency of collective chemotaxis by the regular replacement of chemotactically impaired cells that have been exposed to a high concentration of chemoattractant at the front of the aggregate [9]. Our simulations show that intermittent attachments generate fluidity within a migrating aggregate, so that individual cells are able to move between the front of the aggregate, where their polarity is highly biased and nutrients are expected to be plentiful, and the back of the aggregate, where their bias is low and nutrients are expected to be scarce (Fig. 3E, Fig. S3F). When this fluidity is absent – for example, for very small values of the attachment time T_a, for which cell rearrangements are unable to occur (Fig. S1B) – we find that a solid-like aggregate migrates in the direction of bias (Movie S4). Overall, these results suggest that intermittent attachments generate an emergent cell motion that causes the rearrangement of cells within migrating aggregates, with significant consequences for biological outcomes in individual cells.

D. Emergent material properties

To quantify more precisely the link between intermittent cell interactions and the observed fluid-like properties of surface-associated cell aggregates, we performed idealized simulations to extract the properties of cell aggregates. First, we measured aggregate surface tension by simulating aggregates squeezed between two fixed plates that are non-adherent to the cells. Owing to emergent surface tension, the aggregate's preferred shape is spherical; the confinement therefore induces a net outward force F on the plates. We measured this force computationally and computed the two principal radii of curvature of the aggregate R_1 and R_2 (Fig. 4A), allowing us to calculate an effective surface tension Σ using the established formula [25, 49]

$$\Sigma = \frac{F}{\pi R_3^2}\left(\frac{1}{R_1}+\frac{1}{R_2}\right)^{-1}, \tag{5}$$

where R_3 denotes the radius formed by the contact area of the aggregate with the plates (Fig. 4A). Our simulations predict that a higher attachment strength increases effective surface tension (Fig. 4B), in line with results from passive systems [50]. We found that a reduction in attachment time generally increases surface tension (except at very small values of T_a), which suggests the average cell-cell interaction force is stronger if new attachments are more regularly being formed, so that the springs in the model are generally more extended (Fig. 4C). In general, these simulations predict that intermittent attachments generate a quantifiable surface tension in cell aggregates which increases with attachment strength, a prediction that is testable in future experiments with a similar protocol.

Second, to measure the contribution of intermittent cell-cell attachments to emergent tissue fluidity, we performed simulations of unconfined cell aggregates with periodic boundary conditions (Fig. 4D). We extracted the mean-squared displacement (MSD) of cells from their initial locations. The MSD was found to increase approximately linearly with time (Fig. 4E), suggesting an emergent diffusion of cells caused by intermittent cell-cell attachments. We therefore quantified the fluidity of the cell aggregate by extracting the effective diffusion coefficient D [51] by fitting a straight line to the MSD vs time plot using the relationship

$$D = \frac{\langle \Delta r^2 \rangle}{6t}, \tag{6}$$

where Δr denotes the displacement of a cell, t indicates time and $\langle \cdot \rangle$ denotes an average across the aggregate. Our results show that, whereas the diffusion coefficient D monotonically increases with F_a (saturating for large F_a; see Fig. 4F), it has a non-monotonic dependence on attachment time T_a; initially, D increases with increasing T_a, but it decreases with T_a when T_a is above a critical value. These results are broadly in line with experimental measurements which show that drug-resistant OVCAR-3 aggregates exhibit greater fluidity than drug-sensitive aggregates (Fig. S4A), given our prediction that the drug-resistant cells attach to each other more strongly (see Fig. 2B and associated discussion in Section III B). Overall, our simulations predict that intermittent cell-cell attachments generate a quantifiable emergent fluidity in cell aggregates, which is controlled by the strength and intermittency of the attachments.

Physically, we expect emergent diffusion to arise from a combination of "active" movement from attachments and cell rearrangements caused by the active movement

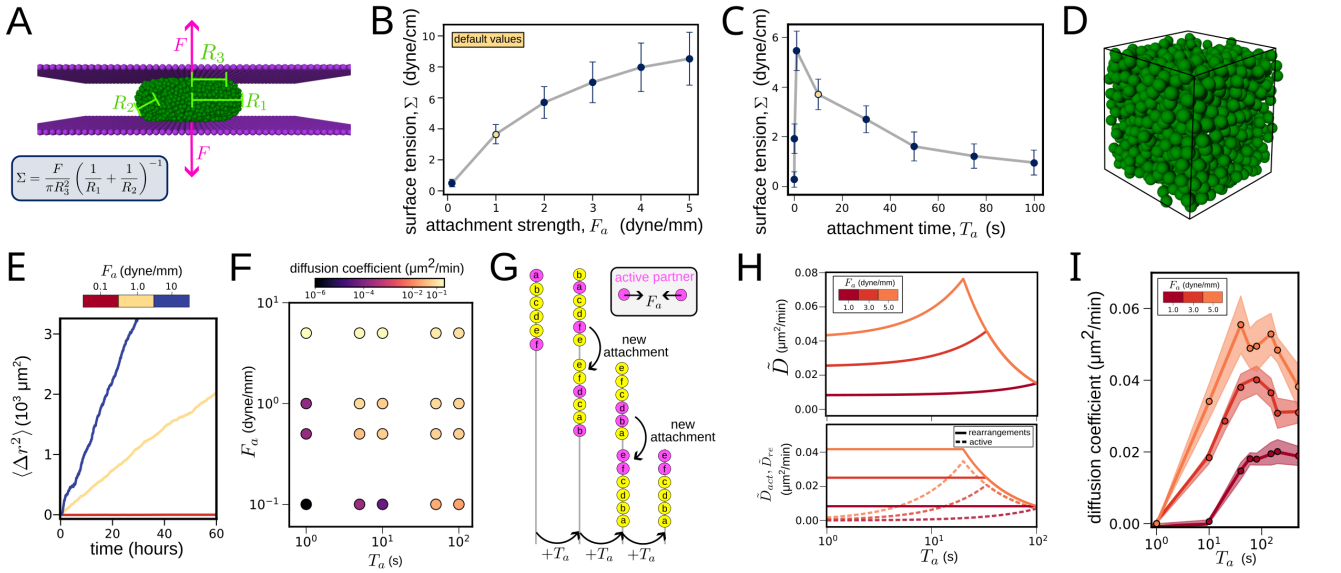

FIG. 4. **Intermittent attachments generate quantifiable emergent surface tension and fluidity of cell aggregates.** (A) Schematic of the parallel plate compression test, where the two walls are assumed to be non-adherent to the cells, unlike the simulations in previous sections. (B,C) Variation in emergent surface tension with attachment strength F_a (B) and attachment time T_a (C), for the default parameter set mentioned in the Supporting Information. (D) Schematic of the simulation used for quantifying the emergent fluidity. The black lines denote periodic boundaries. (E) Mean square displacement vs. time for different values of attachment strength when the attachment time $T_a = 10$s. (F) Variation in emergent diffusion coefficient for different values of attachment strength and attachment time. The colorbar denotes the value of the diffusion coefficient in the units of μm^2/min. (G) Schematic of the simplified one-dimensional model used for gaining insight into the emergent fluidity. We consider a simplified 1D model where cells actively attract one another, and other cells that lie in their path undergo rearrangements. More details on the model can be found in the Supporting Information. (H) Emergent diffusion coefficient as a function of attachment time T_a, for different values of attachment strength F_a, as predicted from the reduced 1D model. The top panel depicts the total diffusion coefficient; the bottom panel shows the contributions from active attachments and associated rearrangements. (I) Emergent diffusion coefficient from 3D cell-based simulations.

of other cells (Fig. S4B). To explore in detail the importance of each type of motion, we built a simplified 1D on-lattice model in which cells move via intermittent active attraction to other cells, while displacing the cells between them (Fig. 4G, Supporting Information). The simplified 1D geometry allows us to obtain an analytical expression for the emergent diffusivity $\tilde{D}$ (see Supporting Information) that can be decomposed into an active part $\tilde{D}_{\rm act}$, arising from the active attachment of a cell, and an accompanying rearrangement part $\tilde{D}_{\rm re}$ that arises from rearrangements due to the active motion of other cells,

$$\tilde{D} = \tilde{D}_{\rm act} + \tilde{D}_{\rm re}. \tag{7}$$

We find that

$$\tilde{D}_{\rm act} \sim \sum_{\substack{i\in\{\text{allowed partner}\\ \text{attachment distances}\}}} T_a^{-1} d_i^2(F_a T_a), \quad \tilde{D}_{\rm re} \sim \sum_{\substack{i\in\{\text{allowed partner}\\ \text{attachment distances}\}}} T_a^{-1} d_i(F_a T_a), \tag{8}$$

where d_i is the typical distance (in cell diameters) traveled during an active attachment between cells that are initially i cell diameters apart (see Supporting Information). Surprisingly, this implies that, in this simplified model, for our typical parameter choices, the cell rearrangements contribute more to the emergent diffusion coefficient than active motion caused by attachments (Fig. 4H). However, as the attachment range increases, active motion can have a dominant contribution to $\tilde{D}$ (Fig. S4C).

In terms of overall fluidity, in the 1D model, the typical distance traveled during an attachment d_i (for $i > 1$, i.e. non-adjacent cells) increases with an increase in the attachment strength F_a multiplied by the attachment time T_a, saturating at large $F_a T_a$; see Supporting Information. This is because when $F_a T_a$ is sufficiently large, attachments last long enough for active cell partners to come together and remain attached while being stationary. This means that an increased attachment strength F_a generally increases the emergent diffusion coefficient $\tilde{D}$, in line with simulations (Fig. 4F,I). By contrast, Eq. (7)-(8) imply that an increased attachment time T_a increases emergent diffusion only at small values of T_a, whereas for large T_a an increased attachment time causes a decrease in both $\tilde{D}_{\rm act}$ and $\tilde{D}_{\rm re}$. We therefore predict that tissue fluidity will be promoted if cells form a new attachment as soon as a previous attachment ceases to cause them to move. Overall, these results demonstrate that both ac-

tive attachment-induced motion and cell rearrangements are required for emergent tissue fluidity – each type of motion enables the other, and each contributes significantly to the overall emergent diffusivity of cells.

IV. CONCLUSION

Here, we have used a minimal cell-based model to demonstrate how intermittent cell-cell and cell-surface attachments cause the self-organization of cell populations into 3D aggregates with emergent fluid properties. Our approach – an off-lattice cell-based model in which cells move via intermittent interaction forces – allowed us to capture how dilute, surface-associated cell layers transition to dense 3D aggregates with extensive cell rearrangements. By extracting and analyzing the motion of all cells within aggregates, we have established and quantified how intermittent attachments generate emergent aggregate properties. In particular, we have found that cell aggregates resemble fluid droplets, which tend to minimize their surface area through surface tension. Within aggregates, cells undergo random motion owing to an effective diffusivity (which is much larger than thermal diffusivity) due to active motion and associated cell rearrangements enabled by intermittency in cell-cell attachments. We have shown that our model is able to capture the dewetting of aggregates of MDA-MB-231 human breast cancer cells [10], wettability and shape fluctuations of drug-resistant OVCAR-3 cancer cell aggregates [38], and the chemotaxis of swarms of *D. discoideum* [8]. We have thereby predicted how these biological processes emerge from intermittent cell attachments.

Our model provides a coarse-grained description of the complex processes through which cells physically interact with their surrounding environment. The model is readily extendable to include further biological processes that have been found to impact self-organization, such as cell deformability [52–55], chemotactic chemical communication between cells [56], cell growth and division [57], and genotypic and phenotypic heterogeneity within multicellular aggregates [58]. Broadly, our modeling framework can be applied to understand how cells dynamically regulate their physical interactions with their environment, to tune collective dynamics at the functionally relevant scale of multicellular assemblies.

METHODS

Model for intermittent attachments

The model for intermittent cell-cell and cell-surface attachments works as follows. Each cell can potentially form an attachment with any other cell that lies within an annular region, which is demarcated by an inner radius (r_1) denoting the effective size of the cell, and an outer radius (r_2) corresponding to the maximum length of a protrusion (Fig. 1C). To form an attachment, the cell randomly chooses another cell (or fixed surface sphere; see Fig. 1C,D) within this region; each cell within the attachment distance has an equal probability of being chosen (unless the direction of attachment is biased by chemotaxis; see Supporting Information). When a cell forms an attachment to another cell or surface sphere, it forms a spring which pulls the two cells towards each other with force given by Eq. (2) and Eq. (3) for cell-cell and cell-surface attachment forces, respectively. For cell-surface attachments, the surface sphere is not allowed to move. During the course of an attachment, if the distance between the two cells is less than or equal to r_1, the attachment force becomes zero, and if the distance becomes greater than r_2, the attachment is broken. Each spring-like force remains active for a duration of time that, on average, is equal to T_a, before the attachment disappears and another is instantaneously formed using the same algorithm. The duration of each attachment is sampled from a Gaussian distribution centered around T_a, with variance T_v, which is introduced to account for the variability in the timescale for cell-cell and cell-surface attachments that is observed in single-cell experiments [59].

All cells can form only one attachment or protrusion at any given time, but any cell can exchange forces with multiple other cells due to its own protrusion and those of other cells. Computationally, at any given point in time, we define the set of interaction partners (P_i in Eq. (2)) of each cell as all the cells that are exchanging mutually attractive forces with the cell. Despite the force between two attached cells being represented as the derivative of a harmonic spring potential (Eq. (2)), the total force experienced by a cell cannot be expressed as the derivative of a scalar potential. This is because the intermittency in cell attachments does not obey the conservation of mechanical energy; in practice, this is because the spontaneous cell polarity and subsequent force generation by the actin cytoskeleton arise from the chemical machinery of the cell. Note that the equations of motion of cells in our model, given by Eq. (4), do not include any noise term, which means that any kind of diffusion seen in the simulations emerges from the intermittent cell-cell attachments. Stochasticity in the model is caused by the random choice of other cells when a cell forms a new attachment.

Parameter estimation

We estimate both the attachment force F_a and average attachment duration T_a from single-cell experimental data. Specifically, we estimate F_a from previous traction force microscopy measurements [39], and T_a from previous experiments analyzing the trajectories of individual cells [60, 61] and imaging the dynamics of individual protrusions [59]. We estimate the value of the mobility parameter μ in Eq. (4) by computing the ratio of

cell speed and magnitude of traction force from single-cell experimental data. The values of these, and other model parameters, including explanations of how they are estimated, are given in Table S1 in the Supporting Information.

When simulating our cell-based model, we consider the model parameters to be identical across all cells within the population, i.e. the population of cells is assumed to be phenotypically homogeneous. Therefore, we do not consider examples such as leader-follower dynamics observed in wound healing [62, 63], where the dynamics are shaped by phenotypic heterogeneity within the population. We also neglect the effects of cell growth and division, which means that the total number and volume occupied by the cells is conserved in all the simulations.

DATA AND SOFTWARE AVAILABILITY

All data are contained within the manuscript. Source code and initial conditions to reproduce simulation results are freely available at https://github.com/dpp98/Interm-attach-cells-3D

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